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DISTRIBUTION BY THE SAP STREAM OF SPORES OF THREE FUNGI THAT INDUCE VASCULAR WILT DISEASES OF ELM [1]

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INTRODUCTION

Several factors common to the two native vascular wilt diseases of the American elm (*Ulmus americana* L.), induced respectively by *Verticillium dahliae* Kleb. and *Dothiorella* (*Cephalosporium*) *ulmi* Verrall and May, and particularly conspicuous in the introduced Dutch elm disease, induced by *Ceratostomella ulmi* Buisman, suggest that in these diseases the wilt-inducing fungi are distributed within the tree chiefly by drifting spores rather than by mycelial growth in the vascular elements. Thus, the discontinuous pattern of the discoloration induced by these fungi in the infected vascular elements suggests distribution by randomly scattered spores rather than by an uninterrupted mycelial growth. Histological studies of the Dutch elm disease reported by Wollenweber (*38*, *39*),[3] Buisman (*9*), Tubeuf (*36*), Banfield and Smith (*4*), and Clinton and McCormick (*11*) disclose a fragmentary, discontinuously distributed, sparse mycelium in the tracheae of infected trees. Conidia have been reported by Wollenweber (*39*) to be produced and randomly scattered in such vessels; conidia and yeastlike cells have been reported by Banfield and Smith (*4*).

The present investigation was designed to ascertain whether spores of the three fungi known to induce vascular wilt diseases of elm are distributed by the sap stream and, if they are so distributed, to show the extent of and the approximate time required for such distribution at various seasons of the year.

Ceratostomella ulmi was used mostly, because (1) it produces spores in enormous numbers in culture as a result of yeastlike multiplication; (2) it can be isolated from infected tissue with less difficulty, owing to the more rapid growth it makes from cultured tissue, than *Cephalosporium* or *Verticillium;* and (3) it is more readily isolated and identi-

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[3] Italic numbers in parentheses refer to Literature Cited, p. 679.

fied by virtue of the coremial fructifications to which it gives rise directly on wood or on cultured tissue.

Preliminary reports of this work have been presented before the American Phytopathological Society (*2, 3, 4*).

REVIEW OF LITERATURE

Wollenweber (*39, pp. 2–3*) in 1929 said of *Ceratostomella ulmi* in the elm:

> Der Pilz lebt hauptsächlich in den der Wasserbewegung dienenden Gefässen des Splintholzes. Er durchwächst dieselben jedoch nur spärlich mit seinen Fäden. Vielmehr geht er so leicht zur Fruktifikation über * * * die bei hefeartiger Sprossung besonders schnell erfolgt, dass er sich mit seinen Konidien vom Saftstrom in der Pflanze passiv treiben und dadurch überallhin im Baume verschleppen lassen kann.

See also Wollenweber and Stapp, 1928 (*41, pl. 2, figs. O and P*).

May (*22, p. 13*) in 1934 reported spores of *Ceratostomella ulmi* to have been passed through "3 feet of elm branch in a few minutes," and from the speed with which the organism moved upward in his inoculations (8 inches per day) he too surmised that "such rapid extension can scarcely be due to simple mycelial growth, but * * * is more likely due to the dispersal of free spores in the water-conducting vessels."

Tubeuf (*36, p. 167*) in 1935, though fascinated by such a singular mechanism, searched the literature in vain for proof, and said:

> Ich habe vergeblich in der Literatur den Nachweis dieses enizigartigen Vorganges gesucht und kann mir die wasserarmen, verstopften und leitungsunfähig gewordenen Tracheen gar nicht zu der ihnen zugeschriebenen Rolle geignet denken.

On March 10, 1936, A. L. Smith [4] demonstrated the presence of spores of *Ceratostomella ulmi* in drops of water that dripped from the base of a 30-foot section of the bole of an American elm 7 inches in diameter breast high, to the upper end of which he had attached, 70 minutes earlier, a reservoir of spore suspension of this organism.

Buisman (*10*) in 1936 sucked spores of *Ceratostomella ulmi* through stems of various elm species with the Melhus "fluometer" by vacuum pressure and air pump. The maximum stem length through which she passed the spores was 88 cm., in *Ulmus elliptica;* the minimum length was 29 cm., in *U. foliaceae* No. 24, the resistant variety now called Christine Buisman. Two days after injection of spores with a hypodermic syringe she recovered the organism from stem tissue as much as 70 cm. above injection points on seedling trees of *U. americana*; but from branches that were injected in this manner immediately after excision from trees of this species, the maximum distance at which she recovered the fungus 2 days after injection was 15 cm. She concluded (*10*) that the water stream in the plant certainly plays a role in the spreading of the disease and assumed that the spores were carried along by the water stream in the plant.

Went (*37*) in 1937, using a similar injection-isolation technique, reported recovery of *Ceratostomella ulmi* 20 cm. below injection points on seedlings of *Ulmus americana* and *U. glabra*, and 30 cm. below injection points on branches of the less susceptible *U. pumila*, 2 days after injection. Records of the length of the stem axes injected are not reported by Buisman or Went.

[4] Unpublished records in the laboratory of the Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, at Morristown, N. J.

Radulescu (*29, p. 631*), who stepped elm branches cut under water in spore suspension, said:

> Nach 14 Stunden konnte durch Abimpfen auf Agar sowie durch mikroskopische Untersuchung festgestellt werden, dass die Konidien auf eine Strecke von 50 cm von der Schnittfläche in den Leitungsbahnen aufgestiegen waren.

The length of the branch is not given. He hung a similar branch upside down, connected the cut end to a reservoir of spore suspension by rubber tubing, then cut off the ends of small twigs and leaves. He reported that *Ceratostomella ulmi* was cultured from drops of water that emerged from certain cut twigs up to 55 cm. from the injection surface, and that 2 months later (*29, p. 633*) "waren im jüngsten Jahrring die für die Ulmenkrankheit kennzeichnenden braunen Steifen längs der Tracheen zu sehen."

Radulescu finally held the tips of excised and unexcised branches under a spore suspension. The 30 to 35 submerged leaves of the branch tip were (*29, p. 634*)—

> bis etwa zur Hälfte abgeschnitten, wodurch die Leitungsbahnen der Blatternerven geöffnet wurden. * * *
>
> Nach 16 Stunden wurde die Untersuchung vorgenommen. Mikroskopisch wurden fast in allen Leitungsbahnen des letzten Jahrringes Konidien beobachtet. Ausserdem wurden an verschiedenen Stellen kleine Holzproben auf Agar abgeimpft.

Positive isolations are recorded throughout all parts of the diagram of this 2.8-m. branch, even to the base. Positive isolations were made only from the "juengsten Jahrringe der Triebe und Blattnerven angegeben."

Radulescu also cut under spore suspension leaves on the tips of the terminal branches of standing trees and held them under this suspension for 6 days. Isolation studies then made revealed the presence of the organism, according to diagrams presented, to the tips of small branches at the base of the two trees studied, respectively 4 and 8 m. in height.

In similar studies on oak and birch, Radulescu recovered the organism at 32 and 40 cm. respectively from leaf injection points 7 days after injection, and at 30 cm. from leaf injections on beech 17 days after injection.

MATERIAL AND METHODS

Spores of the three elm wilt-inducing fungi (*Ceratostomella ulmi*, *Dothiorella* (*Cephalosporium*) *ulmi*, and *Verticillium dahliae*) were injected or inoculated into the bases or tops of tall *Ulmus americana* and *U. fulva* Michx. trees at various seasons of the year. The distribution of these fungi was then traced from the presence of spores in sap samples taken from representative points throughout the injected trees or from the discoloration induced in the tracheae of the trees. Evidence concerning the probable speed of distribution was obtained by observation of the movement of highly colored suspensoids and stained yeast cells, which were injected into similar trees immediately after the removal of the bark.

Only trees in good vigor, which displayed no foliar symptoms of disease, were used. In view of the eradication program, special care was taken to see that none of the trees used experimentally became a hazard for the spread of the disease and all were promptly destroyed after use.

INJECTION PROCEDURE

Large volumes of spore suspension, chiefly of *Ceratostomella ulmi*, were injected into the bases or tops of *Ulmus americana* and *U. fulva* trees 10 to 67 feet high, before, during, and after the leafy seasons of 1936 to 1939, inclusive. The spores were injected through chisel cuts made under the surface of aqueous suspensions held around the tree at the point of injection by means of cone-frustrum pans (fig. 1). The

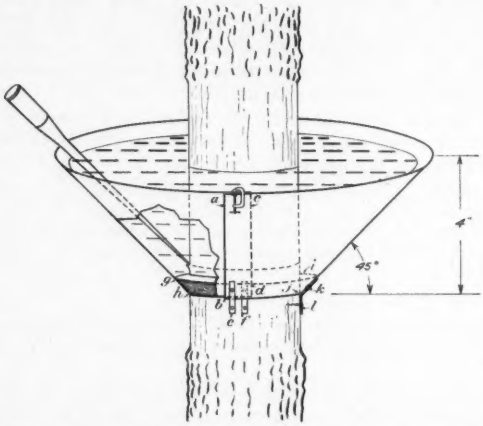

FIGURE 1.—Tree-injection pan (45° cone frustrum 4 inches deep): *ab* and *cd*, Free ends of pan; *e* and *f*, nails fastening ends of pan to stem; *ghij*, layer of grafting wax (or plastic clay) sealing lower edge of pan; *kl*, metal strap fastening pan to stem.

chisel cuts were made to a depth of ¼ to 1½ inches around the entire circumference of the tree at the point of injection. According to Huber (*21*), the vessels of the outermost ring are the most active in conduction. All of these vessels were injected by this procedure. The injection cuts were kept covered by spore suspension throughout the duration of the injection. The injection time varied from several minutes to several days. The suspensions used contained between 1 million and 5 million spores per cubic centimeter. One hundred cubic centimeters to 89 liters of suspension was taken up by individual trees in injection periods of 20 to 120 minutes.

The injection pans used to introduce large quantities of spore suspension into the trees were 45° cone frustrums, 4 to 6 inches deep, ranging from 3 to 20 inches in diameter at their smaller ends (fig. 1). Any stem within the diameter range covered could be fitted by the series of sizes, each being 1 inch larger than the one preceding it. Each pan was cut in one piece from a sheet of 28-gage sheet iron. At its lower end metal straps (*kl*) were riveted and soldered to the outer face of the pan. The pan was fastened by nailing these straps to the stem. The free ends of the pans (*ab*, *cd*) when pulled apart permitted the pan to be slipped around the stem of standing trees. One end (*cd*) was fastened at its lower edge to the stem by a nail at *f*; the other end overlapped and was pulled tight around the stem and was fastened

to the tree by a nail at *e*, to the inner face of the pan at *cd* by a strip of adhesive tape, and at the upper edge of the pan (*ac*) by a C-type screw clamp. This type of pan and injection procedure were used by Shevyrev (*31*). A less satisfactory type of pan, used in the early part of this work, was suspended by wires from nails and had a flexible rubberized cloth cemented to its base. The cloth was bound at its base to the stem by adhesive tape (fig. 2, *A* and *B*).

The pans were sealed to the tree and disinfected by the alcohol-flame procedure prior to each injection. The outer bark at the injection point was shaved smooth to facilitate sealing. The crevices usually present at the lower end of the pan, because of uneven curvature of the stem, were sealed with a layer of grafting wax or plastic clay (fig. 1, *ghij*). The latter was by far the more satisfactory. So sealed, the inner face of the pan and that portion of the stem from which the outer bark had been removed were flamed with alcohol. This provided adequate disinfection of surfaces with which the pure-culture spore suspension subsequently came in contact.

Spores suspended in a few drops of sterile water were injected through cuts made at the tops or bases of similar trees with a 1-inch chisel. The cuts were made deep enough to insure penetration of the outer ring of vessels. Drops of spore suspension were placed on the injecting chisel in such a manner as to come in contact with the severed vessels as the chisel was withdrawn. Additional drops of spore suspension were added until the cuts had absorbed one-half of a cubic centimeter of the suspension, or during the dormant season, until the chisel-cut crevices were filled.[5]

INOCULATION PROCEDURE

Similar trees were inoculated. Chisel cuts were made at one or several points in the trees to a depth which insured that the outer ring of conducting vessels would be severed. Five to ten minutes later the cut surfaces were brushed with a camel's-hair brush, which just previously had been moistened, but not saturated, with spore suspension. It was assumed that spores would not be introduced into the functional vessels by this procedure. Results indicated that none were. Spores can be distributed by the sap stream in trees so treated only after the hyphae that arise from the inoculum have penetrated into functional vessels and have there released new conidia (*4*).

TREATMENT OF TREES AFTER INOCULATION OR INJECTION

The experimental trees were felled 15 minutes to several months after spores had been introduced into them. Those felled within 3 weeks after inoculation or injection were immediately defoliated and sectioned if spore distribution was to be traced by the discoloration procedure. They were cut at roughly one-third, two-thirds, and five-sixths of their height. They were then left lying in the shade, usually on the ground where they fell, until the discoloration induced by the spores developed. Spore distribution in trees felled 21 or more days after injection or inoculation was usually traced immediately by the discoloration procedure, and the trees were then destroyed. The leaves were not removed from such trees.

[5] During the dormant season, water was drawn into such cuts so slowly that it was frequently impracticable to stand by until one-half of a cubic centimeter of suspension had been absorbed. During the leafy season, one-half of a cubic centimeter was usually absorbed within a few seconds from a quarter-inch chisel cut on elm.

FIGURE 2.—*A* and *B*, Injection pans in place at the base and at the top of the boles of American elms; *C*, streaks of discoloration produced on the surface of the wood of a diseased elm by invasion of the new ring of vessels by *Ceratostomella ulmi*; *D*, sections of spore-injected trees in position for displacement of sap and spores by water held in watertight collars at top of sections.

When spore distribution was to be traced by the sap-displacement method, trunk and leader branches were cut into 3- or 4-foot lengths and taken directly to the laboratory. The small leafy branches and twigs were then lopped off with a knife or a hatchet. Before cutting, the stem surfaces and implements were alcohol-flamed. Immediately after cutting, the cut ends were painted with or immersed in a hot vaseline-paraffin mixture as protection against the introduction of contaminants.

METHODS OF TRACING DISTRIBUTION OF SPORES IN THE ELM

Sap-displacement Procedure

The distance to which the spores were distributed in the tree was traced by the sap-displacement procedure from the presence of the injected spores in sap displaced from various parts of the tree.

Sap was displaced with water by gravity from each 3- or 4-foot section of the injected stem. The bark was shaved smooth at the upper end of each section; the wax was cut from the outer several rings of large vessels at this end of the sections; the surfaces were flamed; a sterile metal collar was bound with adhesive tape around the stem over the smoothed bark, and then sealed with grafting wax. The collar attached in this way formed a reservoir to which sterile water was added after the log had been suspended vertically (fig. 2, *D*). Before suspending the logs, their lower ends were pointed with an axe, then alcohol-flamed. Liquid dripped from the lower ends of these logs within a few minutes; it was caught in sterile flasks and later was examined for spores. It was assumed that the water would wash out many spores or would displace such sap and spores as might be in the stem section in those vessels through which spores had been distributed. Usually from 50 cc. to 500 cc. of liquid, depending on the diameter of stem, was collected in this manner from each stem section.

The sap and water displaced from the stem sections were then immediately centrifuged, examined for spores, and cultured. The living cells in the sap samples were concentrated in a 0.2-cc. volume of the liquid by centrifuging. Microscopic observation was made of drops of this concentrate for the spores injected. Spores of *Ceratostomella ulmi* were readily recognized if present in their yeastlike form (fig. 3). Ultimate identification of the spores was obtained from the character of the mycelial growth and fructifications to which they gave rise when isolated on acidified malt agar. To isolate them each concentrated sap sample was diluted with sterile water. Each of three to five Petri dishes containing sterile solidified agar was then flooded with 1 cc. of the diluted sap sample. Proper dilution was computed from the number of spores counted in the concentrated sap samples. Three dilutions were cultured in each case, i. e., the dilution considered optimum and that on either side thereof, in a series of dilutions each 10 times greater than the one preceding it. The plates were examined for colonies of the fungus injected after they had incubated a suitable time. The number of colonies of the injected fungus that appeared in the plates was an index of the number of viable spores of this fungus contained in the liquid displaced from the stem section in question. This in turn was demonstration that the spores injected had been distributed to the horizontal level of at least the lower end of this stem section.

Discoloration Procedure

Several days to weeks after the injection of the spores of any of these fungi into *Ulmus americana* or *U. fulva*, either in the spring or in the leafy season, the vessels occupied by the injected spores showed conspicuous discoloration (fig. 2, *C*). The locus of distribution and the distance to which the spores had been distributed were then apparent from inspection of the vessels. Isolations were made from this discolored tissue at representative points in each tree, in order to confirm the association of the injected organism with the discoloration. This method also served as a further check when the sap-displacement method was employed, since not all the spores introduced by the injection were displaced with the sap.

Figure 3.—Yeastlike cells found in sap displaced from the vessels of trees injected with a spore suspension of *Ceratostomella ulmi* and also in sap displaced from naturally diseased trees. These spores are typical also of those produced in liquid culture by *C. ulmi*. × 1,550.

Discoloration develops, during the spring or during the leafy season, in those functional vessels of American elm to which any one of these organisms has gained access. No exceptions were found during this work. This discoloration is of a light reddish-brown to dark-brown color. It arises in and centers around such points in the functional vessels as become occupied by the fungus. The initial color may be of low intensity but can usually be detected within 3 days after such occupation. This initial color is usually reddish brown, sometimes a faint steel blue. It becomes more intense as time passes. The ultimate color generally is a conspicuous reddish to intense dark brown. The streaks formed (fig. 2, *C*) by such discolored vessels are a characteristic sign of the wilt diseases induced by *Ceratostomella ulmi*, *Dothiorella ulmi*, and *Verticillium dahliae*, respectively.

The extent of the distribution of the injected spores in the vessels was readily determined by inspection for discoloration several days to weeks after injection. Distribution was practically limited to the ring of large spring vessels produced during the season of injection. This ring lies essentially on the surface of the wood during the early weeks of the season, i. e., it is covered by only a thin layer of translucent, unlignified new tissue. After the bark had been removed in the early part of the season, the vessels of this ring that were occupied

by the injected spores appeared as brown streaks, in striking contrast to a background of clear, whitish wood (fig. 2, *C*). Since the intensity of the discoloration induced in the vessels increased up to a certain point as time passed, determinations of spore distribution by this method were more readily and more accurately made 2 to 4 weeks after injection. Invasion of the tissue by organisms that penetrated through beetle galleries or drying bark areas after some time usually induced general tissue discoloration, which obscured the discoloration induced in the vessels by the injected fungus. Since this became progressively more extensive with time, 5 weeks during the leafy season was the maximum period feasible in these experiments for development of significant discoloration. After inspection for discoloration, stem samples were taken from representative points for cultural tests, and the trees were destroyed.

When observed in cross section the vessels occupied by the fungi appeared as small spots of discoloration in otherwise clear wood. (See fig. 7.) The color after a time diffused very slightly into contiguous tissue (fig. 4, *B*). From such discolored vessels the injected fungi were readily isolated. In general, with few exceptions, *Ceratostomella ulmi* cannot be isolated from nondiscolored vessels and tissues.

Isolation of the injected organism from the discolored tissue was accomplished by placing chips of this tissue on acidified potato-dextrose or malt agar. After 3 to 14 days' incubation at 24° C., any of these fungi could easily be identified by their characteristic conidial fructifications and growth. When isolation of *Ceratostomella ulmi* by this technique was difficult because of contaminants, the wood chips were held on moist filter paper incubated at 18° for 3 weeks, at the end of which time examination for coremia was made. Representative coremia were then streaked over the surface of sterile nutrient-agar plates. Colonies typical of *C. ulmi*, with characteristic "dog-ear" conidial formations, were the final criteria for identification of the coremia and of the presence of *C. ulmi* in the tissue cultured.

DISTRIBUTION OF SPORES IN ELMS INJECTED STUMP HIGH

SPORES OF CERATOSTOMELLA ULMI

Trees Injected in Spring Before Maturation of New Ring of Large Vessels

Spores of *Ceratostomella ulmi*, when injected at stump height into native elm trees during the spring flowering period or dormant season, were distributed only a few inches above the points of injection. None were recovered by the sap-displacement method at any distance above the injection points. Sap samples were examined and cultured from all parts of the bole of these trees. Discoloration was induced around certain small vessels of the wood (fig. 4, *D*) of all growth sheaths penetrated by the injection chisel in all trees injected in this manner during the spring and leafy season. Color in this system of vessels did not extend beyond 18 inches above the injection point in any elms at any season in those injection experiments in which the trees were felled within 1 day after injection. *C. ulmi* was recovered

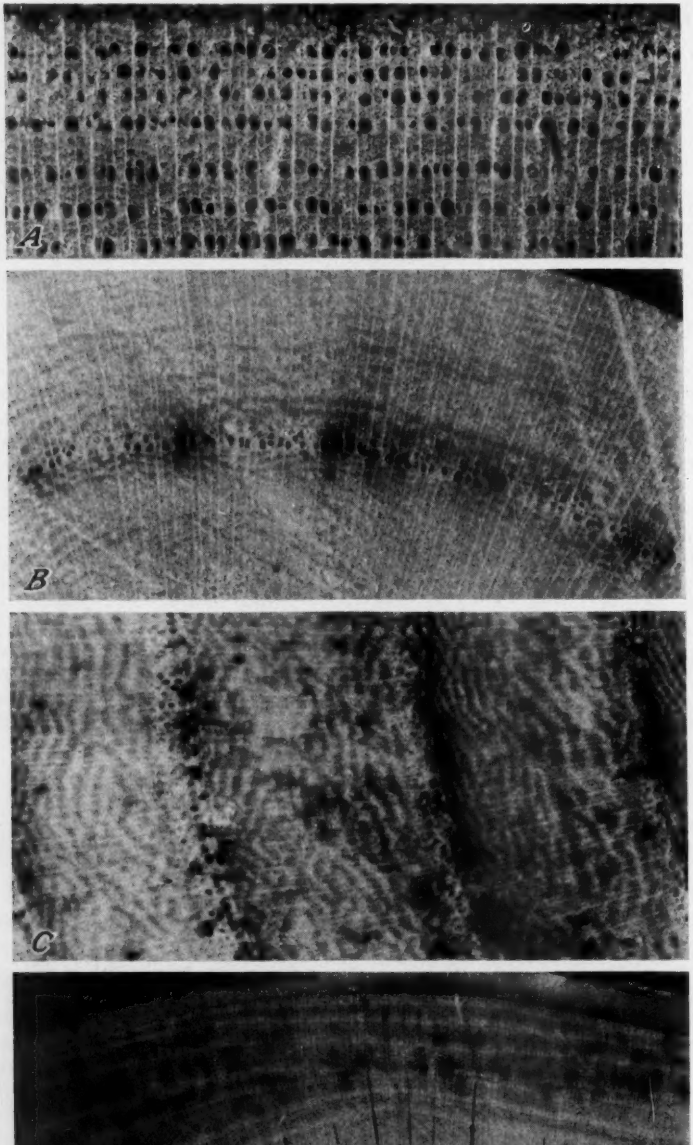

FIGURE 4.—For explanatory legend see opposite page.

from these discolored areas in early trials at only a few inches above the injection point (table 1). It was recovered in later trials up to the limits of the discoloration induced by the spores injected.

Trees Injected in Early Summer or Midsummer

SMALL TREES

Spores of *Ceratostomella ulmi* were injected a few inches above the ground, that is, at stump height, into the main stem of small native elms during the early half of the leafy season. These trees ranged up to 20 feet in height. The injections were made by the usual pan and chisel-cut procedure and ranged from 20 to 60 minutes in duration. The trees were felled and defoliated immediately after injection. Discoloration induced by the spores extended from the injection point up the main stem, and out to within a few feet of the ends of the major branches of the crown (fig. 5, *C*). *C. ulmi* was recovered at representative points up to the limits of discoloration in all the trees that were cultured (table 2). No attempts were made in these experiments to recover the spores from these trees by the sap-displacement method.

TALL TREES

Spores were recovered by the sap-displacement method from the tops of tall elm trees 3 hours after injection during trials conducted in June 1936 (table 1). Three trees, 6 to 7 inches in diameter breast high, were injected 6 inches above the soil line between 10:30 a. m. and 1:30 p. m., June 15, 1936. The weather was ideal for active transpiration in northern New Jersey (temperature, 75° F.; relative humidity, 45 percent; wind, slight). Four and one-half gallons of

EXPLANATORY LEGEND FOR FIGURE 4

A, Transverse section of the bole of tree 116, taken 5 feet above the point of injection with sterile water. Tyloses occlude all inner rings of large vessels. The vessels of the outer ring are clear and show no discoloration. Injected August 3, 1937; photographed February 10, 1938. × 10.

B, Spots of typical discoloration induced by *Ceratostomella ulmi* in the new vessel ring as seen in a representative transverse section of stem (branch G, tree 107) taken at 56 feet above the point of spore injection. Each spot involves one to several vessels. *C. ulmi* was isolated from such spots in nearly all trials, but not from nondiscolored areas. Tyloses have formed subsequent to the injection and occlude practically all vessels of the ring. Injected August 9, 1937; photographed February 10, 1938. × 5.

C, Transverse section of stem taken 35 feet above the point of injection with acid fuchsin from a leafy elm chemically girdled February 1937. The relatively nondiffusible dye remained confined to vessels active in sap flow, i. e., certain small tracheae of the wood in many outer rings. Photographed 1 hour after injection July 16, 1937. × 4.

D, Spots of discoloration induced by *Ceratostomella ulmi* in the bole of an elm injected with spore suspension. Transverse section taken 6 inches above the point of injection. The spots center around certain vessels of the wood in all growth sheaths penetrated by the injection cut. No discoloration developed in the spring ring vessels severed by the injection cut. Vessels of the new ring had not matured. *C. ulmi* was consistently isolated from the discolored areas; it was not isolated elsewhere. The discolored spots did not occur beyond 18 inches above or below the injection point. Injected May 7, 1937; photographed June 1, 1937. × 1.

the spore suspension was taken up by the three trees during the 3-hour injection period. Fifty to one hundred cubic centimeters of liquid was then displaced from the various sections of stem above the injection points (table 1) for trees 8 and 9. Each sample was

TABLE 1.—*Distribution of spores of Ceratostomella ulmi injected* [1] *stump high into American elms at various seasons, 1936*

Tree No.	Injection date	Seasonal condition of trees	Time between injection and sectioning [2]	Tree dimensions	
				Diameter breast high	Height [3]
			Days	*Inches*	*Feet*
1	Apr. 1	Flowering	3	4	22
2	do	do	3	5	18
3	May 1	Leaf buds unfurling	3	5	20
4	do	do	3	5	20
5	Apr. 10	Flowering	28	3	18
6	May 28	Rapid shoot elongation	2	7	35
7	do	do	2	6.5	35
			Hours		
8	June 15	Active growth	3	6	35
9	do	do	3	7	35
92	do	do	3	7	35
10	Nov. 11	Dormant	1	7	35
11	do	do	1	6	35
12	Nov. 6	do	3	7	35
13	Nov. 9	do	3	6	35
14	Nov. 12	do	28	7	35
15	Dec. 2	do	4	8	51
			Days		
16	do	do	10	7	46

Tree No.	*C. ulmi* isolated from sap displaced at indicated distance above injection point [4]							Upper limit [5] of vascular discoloration	Maximum distance above injection point at which *C. ulmi* was isolated
	2–5	5–8	8–11	11–14	17–20	24–27	29–32		
								Inches	*Inches*
1	−	−	−					15	3
2	−	−	−					16	4
3	−	−	−					24	2
4	−	−	−					24	2
5	−	−	−					18	3
								Feet	*Feet*
6			[6] −*		−*	−*		29	28
7			−*		−*	−*		26	26
8			+*		−		+*	32	29
9		+*	+*	+*	+*	+*	+*	30	25
92								32	30
									Inches
10	−	−	−					(6)	6
11	−	−	−					(6)	6
12	−	−	−					(6)	24
13	−	−	−					(6)	12
14	−	−	−					(6)	12
15	−	−	−					(6)	24
16	−	−	−					(6)	12

[1] All vessels of the several outer wood rings were injected with spore suspension approximately 18 inches above soil line.

[2] The interval between the beginning of the injection and the time at which the tree was felled and sectioned into 3- or 4-foot lengths.

[3] Measured from the point of injection.

[4] + indicates that *C. ulmi* was isolated; −, that it was not.

[5] Typical yeastlike spores of *C. ulmi* were observed in the sap samples taken from the injected tree sections designated by an asterisk (*).

[6] No discoloration developed.

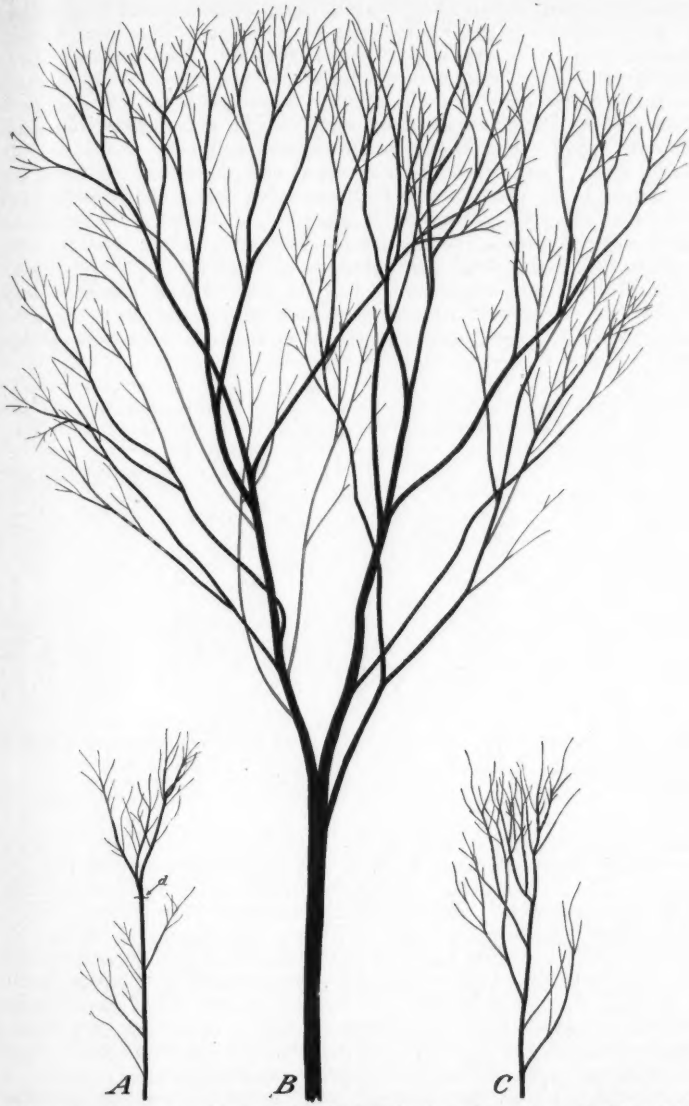

FIGURE 5.—Diagram of spore distribution in American elms: *A*, Tree injected with a spore suspension of *Ceratostomella ulmi* for 20 minutes at point *d*; *B* and *C*, trees injected stump high, *B*, for 2 hours and 20 minutes, *C*, for 20 minutes. All trees were felled and defoliated at the close of the injection period. Shading indicates vascular discoloration with which *C. ulmi* was found associated several weeks after injection. Broken shading indicates discontinuous or interrupted streaking of vessels at limits of spore distribution. All trees drawn to scale 0.5 cm. = 1 foot. Lower 17 feet of trunk of tree in *B* not shown.

then centrifuged, its suspended cells being concentrated in a volume of 4 cc. Typical yeastlike colonies of budding spores (fig. 3) were observed in every sap sample collected from tree 9 and in the samples taken from the lower part of the bole and the top or leader branch of tree 8. That most of these cells were spores of *Ceratostomella ulmi* was established by isolation and growth of the spores on acidified malt agar. Four hundred and eighty colonies of *C. ulmi* developed from 1 cc. of the concentrated sample of the sap that had been displaced from a stem section cut from tree 9 at from 17 to 20 feet above the injection point. Eighty colonies per cubic centimeter developed from sample 24–27, tree 9; 200 per cubic centimeter from sample 29–32, tree 9; 3 per cubic centimeter from sample 29–32, tree 8; and an indeterminate high number from all others, except sample 17–20, tree 8, in which no *C. ulmi* colonies could be detected in the mass of contaminating fungi that overran all plates of this series.

TABLE 2.—*Distribution of spores of Ceratostomella ulmi in small American elms injected stump high in early summer, 1938*

Tree No.	Injection date	Spore suspension injected [1]	Tree dimensions		Maximum height [2] of vascular discoloration	Maximum height [2] at which *C. ulmi* was isolated
			Diameter breast high	Height [2]		
		Cc.	*Inches*	*Feet*	*Feet*	*Feet*
4 [3]	June 3	125	1¼	10.4	8.7	8.7
5	do	200	1⅜	11.1	9.3	9.0
6	June 10	130	1½	11.9	9.1	9.1
10	June 22	475	2½	13.7	12.3	12.3
11	do	1,750	3½	20.0	19.0	19.0
12	July 17	280	1⅜	11.7	7.5	7.4
13	do	300	1½	11.8	8.3	8.3
14	do	300	1¾	13.7	12.2	12.2
15 [4]	do	450	1¾	12.1	9.9	9.6

[1] The injection period was 20 minutes to 1 hour in all cases. All injections were made between the hours of 10 a. m. and 3 p. m. on clear days.
[2] Measured from the point of injection.
[3] See figure 5, *C*.
[4] See figure 6, *A*.

Confirmation of this rapid upward distribution of spores in these trees during the 3 hours of the injection was obtained from the discoloration that subsequently developed in the outermost or new ring of large spring vessels. This discoloration was well developed within 10 days after injection and extended as a few streaks to 32 and 30 feet, respectively, in trees 8 and 9, and to 32 feet in tree 92, from which the sap was not displaced. Discoloration induced by *Ceratostomella ulmi* was as abundant in the stem sections from which spores were taken by the sap-displacement technique as in those not so treated. *C. ulmi* was recovered from chips of discolored tissue taken from these trees at 29, 25, and 30 feet, respectively, and cultured on agar.

The distribution of *Ceratostomella ulmi* spores in large native elms was studied in more detail by the discoloration procedure. Trees of different height, diameter, and age were injected at the base of the bole by the usual pan and chisel-cut procedure for 20 minutes to 4 hours. Vascular discoloration developed to within a few feet of the tops of most of the trees studied in the early or midseasons of 1937 and 1938. The trees ranged up to 64.8 feet in height. The discolor-

ation in general extended into most of the major branches, into numerous secondary branches, in some instances even into smaller twigs throughout the crowns of the trees. There were major branches in which no vascular discoloration developed, however. In one tree (18 of table 3), the discoloration failed at roughly two-thirds of the height of the tree. With certain minor exceptions, *C. ulmi* was found associated with the vascular discoloration at all levels above the injection points in these trees. On the other hand, all routine attempts to isolate this organism from nondiscolored regions of the vascular system 2 to 5 weeks after injection and felling were negative. Hundreds of such attempts were made over a period of 4 years. The upper limit of spore distribution, as indicated by the discoloration induced, is recorded in table 3. No comparable discoloration (fig. 4, *A*) developed in the vessels of two 50-foot trees injected with sterile tap water under like conditions, and isolations from the outer vascular rings in these trees did not yield any wilt-inducing fungi.

TABLE 3.—*Distribution of spores of Ceratostomella ulmi in tall American elms injected stump high in early summer to midsummer, 1937–38*

Tree No.	Injection date	Schedule of operations			Spore suspension injected	Tree dimensions		Maximum height [2] of vascular discoloration	Maximum height [2] at which *C. ulmi* was isolated
		Injection	Felling [1]	Defoliation completed		Diameter breast high	Height [2]		
	1937	*Clock time*	*Clock time*	*Clock time*	*Liters*	*Inches*	*Feet*	*Feet*	*Feet*
100	Aug. 2	10:50 a.m.	11:55 a.m.	12:38 p.m.	5	8.5	38.0	34.5	32.2
101	do	2:00 p.m.	3:00 p.m.	3:40 p.m.	11	9.0	44.0	42.5	34.0
103	Aug. 3	10:40 a.m.	[3] 11:50 a.m.	12:10 p.m.	14	8.5	49.0	45.0	36.0
69	June 9	1:12 p.m.	[3] 4:00 p.m.	10:30 a.m.	20	9.0	50.0	48.0	48.0
70	do	1:56 p.m.	[3] 3:20 p.m.	11:00 a.m.	14	10.0	50.5	49.0	46.0
71	do	2:40 p.m.	[3] 3:47 p.m.	11:30 a.m.	14	8.0	50.0	46.2	45.0
73	June 15	11:05 a.m.	[3] 12:00 m.	1:05 p.m.	10.5	9.0	50.0	47.7	47.0
95	July 28	12:30 p.m.	1:30 p.m.	3:10 p.m.	20	9.5	58.0	44.0	39.0
68	June 7	2:38 p.m.	3:40 p.m.	4:15 p.m.	20	10.0	60.0	57.5	53.0
94	July 28	10:30 a.m.	11:30 a.m.	12:25 p.m.	14	8.0	60.0	54.0	53.0
99	July 30	12:30 p.m.	1:30 p.m.	2:00 p.m.	33	12.0	64.0	56.0	51.0
107	Aug. 9	11:30 a.m.	1:30 p.m.	4:00 p.m.	42	12.5	64.8	63.7	63.7
	1938								
18	June 30	2:58 p.m.	5:01 p.m.	6:25 p.m.	27	9	53.0	37.5	37.0
19	do	4:00 p.m.	8:00 p.m.	8:30 p.m.	6	6	49.1	47.2	45.0
43	do	9:22 a.m.	[3] 11:22 a.m.	12:00 m.	8	8	55.5	54.0	54.0
44	do	9:39 a.m.	[3] 11:45 a.m.	12:15 p.m.	14	6	53.3	46.8	46.0
45 [4]	do	12:17 p.m.	[3] 2:37 p.m.	3:10 p.m.	11	6	50.0	47.0	46.5
46	do	12:50 p.m.	[3] 2:45 p.m.	3:40 p.m.	12	9	52.0	49.6	49.0

[1] The hour at which the injection pan was removed and felling operations were begun.
[2] Measured from the point of injection.
[3] All trees were sectioned at roughly ⅓, ⅔, and ⅚ of their height after felling, except these trees. These were left intact until examination for discoloration was made several weeks after injection.
[4] See fig. 5, *B*.

The distribution of spores in tree 45, representative of the large-tree class, is diagramed in figure 5, *B*. The heavy shading in trunks and branches indicates the presence of more or less continuous streaks of discoloration induced by the spores injected in the outer vascular ring. In most cases these streaks disappeared in the distal parts of the branches. There, isolated points or dashes of color were usually found separated by a few millimeters to decimeters along the vertical axis of the branch. Such a discoloration pattern in the outer ring of large vessels is indicated by broken shaded lines in the diagram.

Representative areas of this discoloration in the outer vascular ring of trunk and crown were cultured. *Ceratostomella ulmi* was consistently isolated from all such areas, including the isolated spots and dashes of color in the vessels at or below the points where the discoloration disappeared. *C. ulmi* was not recovered in any section in these trees where there was no discoloration in the outer vascular ring.

Further details as to the extent and locus of spore distribution in these trees are illustrated by representative tree 107 in figures 6, *B*; 7; and 8.

Most of the vessels of the outer ring in one of the arms of this twin-leadered tree were discolored at 20, 30, and even 40 feet above the injection point (fig. 8, *E*, *C*, *A*). Relatively few vessels were discolored in the other arm (fig. 8, *B*, *D*, *F*), but again there were roughly as many vessels discolored in it at 40 as at 20 feet above the injection point. The extent of the discoloration in branch G, one of the terminal branches of coleader *E*, *C*, *A*, is portrayed in the photodiagram (fig. 6, *B*). Its base point was 52 feet above the point of injection. Discoloration extended into all of its secondary branches, commonly into twigs three-eighths of an inch or less in diameter. Photographs of the discoloration induced in the vessels of this branch were taken at points indicated by letters (fig. 7). The highest point to which discoloration extended was 63 feet 8 inches above the injection point, 13 inches below the end of this branch. The branch at this point was 3 years old and three-sixteenths of an inch in diameter. *Ceratostomella ulmi* was isolated at many representative points throughout the trunk and crown, even to the uppermost limits of the discoloration indicated in figure 6, *B*. Tree 107, injected at 11:30 a. m., August 9, 1937, took up 42 liters of spore suspension of approximately 3,000,000 spores per cubic centimeter within 1 hour. The injection ended at 12:30 p. m. The tree was felled at 1:30 p. m., and was sectioned at 20, 40, and approximately 50 feet above the injection point by 2 p. m. No discoloration was present at the points sectioned at this time. All branches were defoliated by 4 p. m. It was a codominant member of a close stand of hardwoods on poorly drained soil.

UPPER LIMIT OF SPORE DISTRIBUTION

The distribution of spores that occurred in trees felled in early summer or midsummer, within 20 minutes to 24 hours after injection, generally terminated above the injection point in the same type of growth, regardless of the height of the elms injected (fig. 6) or the height at which injection was made (fig. 5). Thus in tree 107, vascular discoloration from which *Ceratostomella ulmi* could be isolated generally stopped in the terminal branches a few inches or feet below the terminal growth scars formed in the preceding season. This was as much as 63.7 feet above the injection point in this 64.8-foot tree. The branches at these points were 2 to 10 years old, but generally 3 years old or more. In diameter they varied considerably, but usually they were one-eighth to three-eighths of an inch. The fungus was not distributed into all such, to be sure, since some branches, even an occasional major branch, developed no vascular discoloration in this and other trees.

FIGURE 6.—Photodiagram of spore distribution in representative trees injected stump high for 1 hour with spore suspension of *Ceratostomella ulmi*. Shading indicates vascular discoloration with which *C. ulmi* was found associated several weeks after injection; broken lines indicate parts of branching structure where discoloration was not produced and from which *C. ulmi* could not be isolated. Trees felled and defoliated at close of injection period. *A*, Small tree (No. 15) shown entire. *B*, Branch G, from top of 64.8-foot tree 107, excised at 52 feet above point of injection, at close of injection period. Photographs of discoloration induced in vessels, taken at the points indicated by lower-case italic letters, are presented in figure 7. For extent of vascular discoloration at points below 50 feet in this branch, see figure 8, *A*, *C*, and *E*.

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Spores injected July 23, 1937, at 41 feet above the ground in 67-foot tree 86 of table 7 were distributed throughout that portion of the crown supported by the axis injected. The vascular discoloration from which the injected fungus could be recovered terminated in the

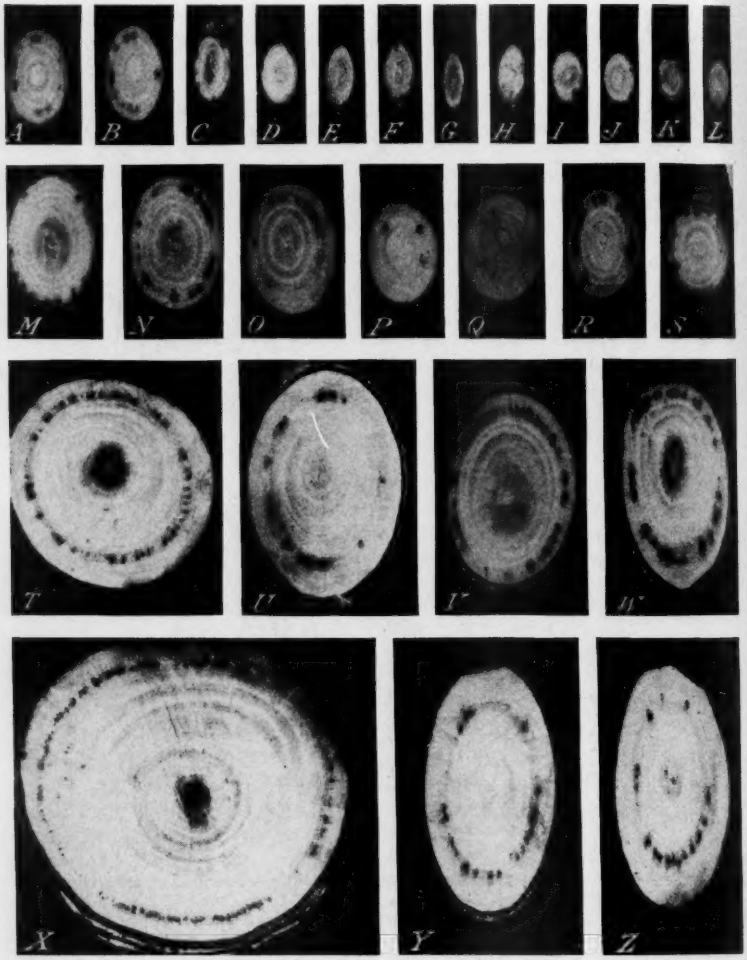

FIGURE 7.—Discoloration induced by injected spores in ring of vessels of current season in branch G, tree 107, as seen in cross sections taken at points indicated by letters as in figure 6, *B*.

same kind of growth 2 feet or more from the 1936 terminal growth scars. In tree 42 of table 7, injected 41 feet above the stump June 28, 1938, it extended in certain cases to within a few inches of the branch tips.

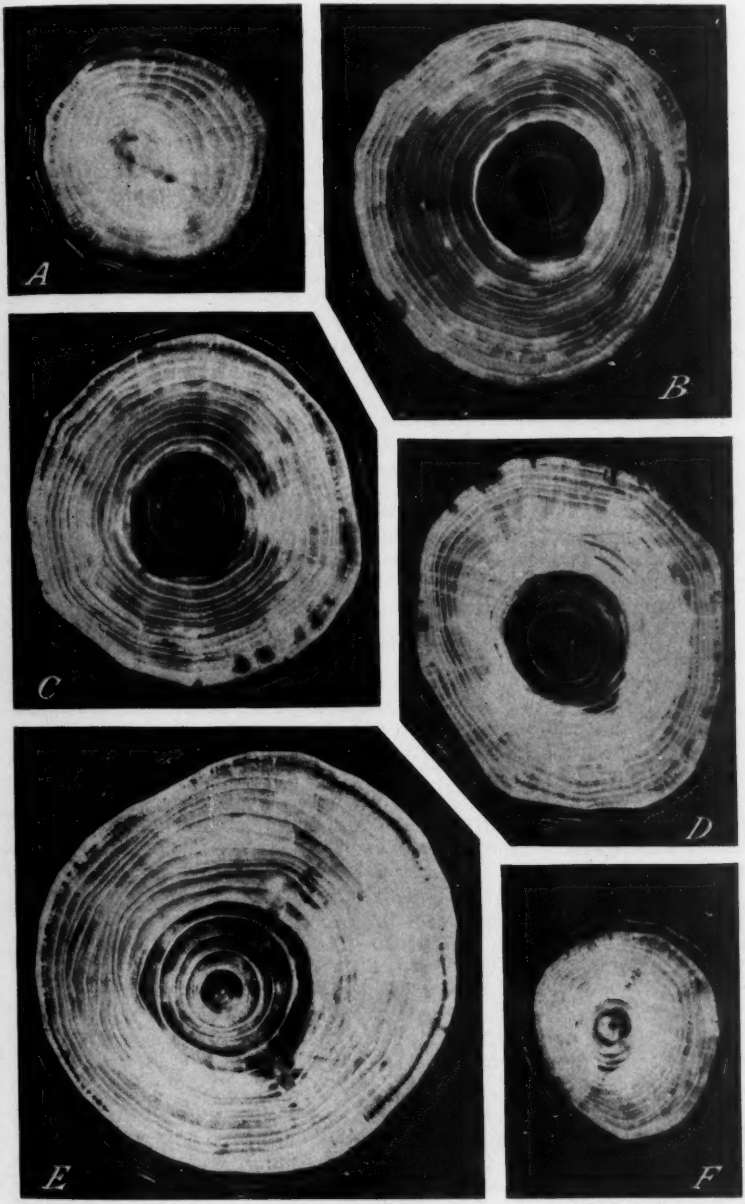

FIGURE 8.—Discoloration induced by *Ceratostomella ulmi* in the new ring of vessels of tree 107 as seen in representative cross sections taken from the two main branches at 20 feet (*B*, *E*), 30 feet (*C*, *D*), and 40 feet (*A*, *F*) above injection point. × ½.

Spores injected into terminal branches 18 to 29 feet above the stump in American elms, at points where the diameter of these branches did not exceed one-half inch, were distributed to within about the same minimum distance from the branch ends as they were in injections made at the base of the crown or lower. Discoloration ended in these terminal branches at or several inches below the terminal growth scars formed at the close of the preceding season. The frequency with which discoloration extended to such points was higher in these terminal-branch injections than in injections made at lower levels.

Vascular discolorations from which *Ceratostomella ulmi* can be isolated, however, in time generally extend into the terminal growth produced during the season of injection. Thus, in the type of injection just described it was commonly present in the current season's growth of the branch terminals 5 days after injection. However, it generally failed in these branches at about 12 inches from the apices of the new growth. Two weeks after injection it still failed generally at about the same distance from the apices of the new growth. Discoloration at this time, however, did extend in one case to the end of such growth in a terminal branch. By the end of the season it was commonly found to the limits of growth throughout the crowns of trees injected before midsummer, but even then it frequently came to an end below the terminus of the growth produced during the season of injection. Although most of the branches and twigs in the crowns of such trees were then invaded by the fungus, there were notable exceptions wherein even relatively large secondary branches were found without discoloration or invasion by the fungus.

Trees Injected in Late Summer or Early Autumn

Spores of *Ceratostomella ulmi* were injected by the usual procedure at stump height into elms of different height in the latter part of the seasons of 1937 and 1939. In general the distribution of spores in these trees was markedly different from that in trees injected in the early part of the season. The locus of distribution was the same, i. e., typical discoloration developed only in the outer ring of large spring vessels, but the distance to which the spores were distributed above the injection point was conspicuously limited as compared with that in trees injected in the early part of the season. Thus, spores of *C. ulmi* apparently were not distributed to roughly more than one-half the height of 10 small trees (less than 25 feet in height) injected in the latter half of the 1937 season (table 4), whereas in the 9 small trees studied in the early half of the 1938 season (table 2) they rose to within an average distance of 2.2 feet of the tips of trees that averaged 12.9 feet in height. The records of spore distribution in tall trees up to 64.8 feet in height display the same trend, but in more striking contrast. The average height of the 18 tall trees injected with spores early in the seasons of 1937 and 1938 (table 3) was 52.8 feet; the average height of spore distribution was 48.3 feet, judged from vascular discoloration induced by the injected spores. The average height of spore distribution in 15 large (greater than 26 feet in height) leafy trees injected in early autumn in 1937 and 1939 was 12.3 feet; the average height of the trees was 38.1 feet. In other words, spores were not distributed to more than an average of three-tenths of the height of trees injected in late season, whereas they were distributed to more than nine-tenths of the height of the trees injected in early season.

TABLE 4.—*Distribution of Ceratostomella ulmi spores in American elms injected stump high in late summer and early fall, 1937 and 1939*

Tree No.	Injection date	Injection time	Spore suspension injected	Tree dimensions		Maximum height [1] of vascular discoloration	Maximum height [1] at which *C. ulmi* was isolated
				Diameter breast high	Height [1]		
	1937	*Hours*	*Liters*	*Inches*	*Feet*	*Feet*	*Feet*
105	Aug. 3	1	3.0	2.0	13.5	6.5	4.0
104	do	1	1.2	2.5	16.0	4.5	4.0
119	Sept. 23	1	2.0	2.0	15.0	6.8	[2]
120	do	1	1.5	2.5	13.0	6.7	4.0
121	do	1	1.5	2.0	14.0	7.5	4.0
126	Oct. 8	1	1.5	2.0	15.0	7.5	6.0
127	do	1	2.5	3.0	18.3	8.0	7.5
102	Aug. 3	1	4.0	4.0	21.0	9.0	8.0
108	Aug. 11	1	8.0	3.0	21.3	8.0	8.0
117	Aug. 23	1	3.0	4.0	23.0	21.5	[3]
128	Oct. 8	2	4.0	4.0	27.0	11.0	11.0
129	do	2	6.0	5.0	32.0	9.0	9.0
123	Oct. 2	3	5.0	4.0	39.0	10.0	9.0
124	do	2	6.0	5.0	45.0	12.0	11.0
122	Sept. 24	2	40.0	10.0	57.0	29.0	29.0
125	Sept. 30	2	89.0	20.0	60.0	30.3	25.0
	1939						
973	Sept. 28	2.0	3.5	4.2	32.5	12.0	12.0
974	do	1.0	4.0	4.5	34.2	12.0	12.0
975	do	1.8	4.6	5.5	37.1	7.3	7.3
976	Oct. 5	[4] 4.0	5.0	4.0	28.5	11.0	11.0
977	do	[4] 4.2	7.0	5.0	35.2	9.8	[5] 9.0
978	do	[4] 4.2	2.9	3.0	28.6	8.2	[5] 8.0
979	do	[4] 3.7	7.3	6.3	39.7	8.6	[5] 8.0
980	do	[4] 4.0	6.3	4.0	39.8	7.7	[5] 7.0
981	do	4.0	4.7	3.7	36.2	6.7	[5] 6.0

[1] Measured from the point of injection.
[2] No isolations were made from this tree.
[3] All attempts to isolate *C. ulmi* from the vascular discoloration recorded failed.
[4] The injection pan was empty when the tree was felled at the end of the period indicated. The exact length of the injection is therefore unknown.
[5] No isolation was attempted at the maximum height to which vascular discoloration extended.

The number of vessels invaded within a few feet of the point of injection in trees injected in the latter part of the leafy season was essentially the same as in trees injected in early season, that is, practically all vessels of the outer ring were invaded and a solid ring of discoloration was produced; but, whereas this more or less complete ring of discoloration usually continued for many feet above the injection point in large trees injected in early season (figs. 7 and 8), the number of discolored vessels rapidly diminished with increasing distance above the injection point in trees injected in the latter part of the season. Thus, in 57-foot tree 122 (table 4), typical of those studied in early autumn, there was a solid ring of discoloration involving practically all vessels of the ring at 2 feet above the injection point. At 5 feet above the injection point there were 496 spots of discoloration, evenly distributed through the ring, each spot involving 1 to several vessels. Ten feet above the injection point there were but 97 such spots; at 20 feet, only 5; above 25 feet, only 1.

There appears to be no definite time at which this transition occurs. The results for spore distribution in trees injected in the early season were consistent (tables 1, 2, and 3), as were those for trees injected in late season (table 4), but those injected in late July or early August, i. e., in midseason, were highly variable. For convenience, the results from trees injected in this transitional period were tabulated either with those from trees injected in early season or with those

from trees injected in late season, according to their conformation to the one group or the other.

Trees Injected During Dormant Period of Late Autumn or Early Winter

Spores of *Ceratostomella ulmi*, when injected stump high into American elms after leaf fall in the autumn, could not be recovered at more than 24 inches above the point of injection. None were detected in sap displaced from sections of the injected stem taken at 2 to 5 feet above the injection point, nor elsewhere throughout these trees. No discoloration subsequently developed in the new ring of vessels or elsewhere in these injected trees, even though the logs into which they were cut were held in the greenhouse at approximately 60° F. until March of the following year. In every case, however, the organism was isolated at from 6 to 24 inches above the point of injection (table 1) by the following procedure. Sections of stem 6 inches in length were taken from the injected trees at respectively ½, 1, 2, and 5 feet above the injection point. Chips to a depth of several rings were taken aseptically from the complete circumference of these sections and cultured on potato-dextrose agar. The cultures were held at room temperature and were checked for the presence of *C. ulmi* 7 and 21 days later.

Girdled Trees Injected During Summer

Spores of *Ceratostomella ulmi*, injected several hours by the usual pan and chisel-cut procedure into two girdled leafy trees, were not distributed more than 16 inches above or below the point of injection. The trees had been girdled the preceding February by application of a band of copper sulfate around the trunk by a technique described by Liming [6] in 1937. These trees consequently produced during the season of injection no new ring of large vessels for a distance of several feet above or below the girdle. The injection of spores was made near the upper limits of the girdled zone. The trees were felled at the close of the injection period. The several outer growth sheaths of stem tissue were generally colored a deep brown by the copper sulfate in the region of the chemical girdle. No vascular discoloration was induced in these discolored areas of tissue by the injected spores. The injected spores, however, induced discoloration in many of the small vessels of all the outer rings severed by the chisel cuts that had not been discolored by the chemical girdle. This discoloration did not extend in these vessels beyond 16 inches. The fungus was recovered from the discolored vessels up to the limit of discoloration, i. e., 16 inches.

Spores of Dothiorella (Cephalosporium) ulmi and Verticillium dahliae

Spore suspensions of *Dothiorella ulmi* and *Verticillium dahliae* were apparently distributed after the manner described for suspensions of spores of *Ceratostomella ulmi* when injected into the bases of trees early in June 1936. The injections were made stump high by the usual procedure. Several cubic centimeters of concentrated spore suspension was introduced into each tree at each of three points around

[6] Liming, O. N. The Dutch elm disease eradication program—objective, methods, and results. U. S. Dept. Agr., Plant Dis. Rptr. Sup. 99: 18–25. 1937. [Mimeographed.]

the circumference. The width of the chisel cuts was 1 inch. The locus and limits of spore distribution were determined from discoloration seen after stripping off the bark when the trees were felled 6 or 7 days later. The discoloration in each instance began at the injection points at the base of the trees and extended up the stem as a series of continuous streaks for various distances, after which the streaks continued in an interrupted pattern—short streaks intercepted by progressively greater distances of clear tissue—until all color disappeared. These fungi did not induce vascular discolorations differing sufficiently to be diagnostic. The discoloration induced by *C. ulmi* was of the same general appearance, type, and color for each organism, i. e., a red to greenish brown of varying intensity, strictly limited to the functional ring of large vessels. It extended to within 1 to 3 feet from the tops of the trees inoculated in this manner with *V. dahliae* and *C. ulmi*, and disappeared at 8 to 11 feet below the tops of trees injected with *D. ulmi* (table 5). The organism injected was recovered in all instances at the limits of discoloration above the injection points and at other representative points in isolations made at the time the vascular discoloration was traced. Only from the trees injected with *C. ulmi* was the injected organism recovered beyond the limits of discoloration. Field observations of these diseases had suggested, however, that the pathogens involved are distributed downward as well as upward, by spores rather than by continuous mycelial growth. The following experiments were therefore undertaken to ascertain whether spores could be distributed downward in elms at various seasons.

TABLE 5.—*Distribution of spores of Verticillium dahliae, Dothiorella ulmi, and Ceratostomella ulmi in American elms, injected*[1] *stump high with spore suspensions of these fungi, in June 1936*

Tree No.	Organism injected	Time between injection and examination	Tree dimensions		Maximum height[2] of vascular discoloration	Maximum height[2] at which pathogen was isolated
			Diameter breast high	Height[2]		
		Days	*Inches*	*Feet*	*Feet*	*Feet*
V6	*V. dahliae*	6	5	32	30	30
V7	do	6	5	28	27	27
V8	do	6	4	23	21	21
D52	*D. ulmi*	7	6	44	33	33
D53	do	7	6	44	33	33
D54	do	7	6	40	32	32
C1	*C. ulmi*	7	7	35	32	32
C2	do	7	6	30	26	29
C3	do	7	6	31	29	30

[1] A few drops of spore suspension were injected into these trees by the usual procedure at one to three points distributed around the circumference of the trunk at one horizontal level 4 to 18 inches above the soil line.

[2] Measured from the point of injection.

DISTRIBUTION OF SPORES OF CERATOSTOMELLA ULMI IN TOP-INJECTED ELMS

INJECTION OF LARGE VOLUMES OF SPORE SUSPENSIONS INTO MAIN STEMS AT BASE OF CROWNS

Large volumes of spore suspensions of *Ceratostomella ulmi* were injected into the main stem of tall polelike elms 13 to 41 feet above the stump at various seasons of the year. The spores were injected through chisel cuts made under a spore suspension held around the

trunk with cone-frustrum pans. All functional vessels in the new-growth sheath at the pan level were injected. The stems in most cases were then cut off some 6 to 10 inches above the pans immediately after the injection was concluded. At varying intervals of time the trees were cut down and sectioned. The maximum distribution of the fungus was then determined by the methods earlier described.

During a 3-day injection period prior to leaf development in the spring, the maximum distribution of the fungus below the injection point was 2 feet. Spores were not recovered. Discoloration, however, developed in the three spore-injected trees. The fungus was isolated from this discoloration at 2, 2, and 1 feet, respectively, below the injection points (table 6).

Spores were recovered at greater distances below the injection points in dormant trees during late autumn and early winter (table 6). The maximum time during which the trees under investigation absorbed the spore suspension probably did not exceed 12 hours; i. e., the injections were initiated at noon, in the case of trees 14, 15, and 16, and the pans were kept supplied with spore suspension until twilight, after which no further additions of spore suspension were made. When the injection was continued for 3 hours or longer, the spores were consistently recovered at 8 to 12 feet below the injection points. There was apparently no further distribution of spores in these trees with the passage of time. Thus, spores were recovered at 8 or 12 feet below the injection points, on trees 12 and 13, 3 hours after the inception of injection. Four or 14 days after the beginning of injection the spores apparently had not been distributed any farther and were recovered again only at 8 and 12 feet, respectively, below the injection points.

No discoloration developed in these trees during observations made between December 2 and January 15, even though the stem sections were held at about 60° F. in the greenhouse. Confirmation of the limits of distribution of the spores was obtained in each instance, however, by culturing chips from the complete circumference of the stem at various distances below the injection point.

Ceratostomella ulmi was recovered from the stem at the ground level within 2 days after top injections of a spore suspension of the fungus into trees during the leafy season, within a few weeks of the time of autumn defoliation. The injections were made at 21 to 34 feet above the stump (table 6). Injection in most instances was initiated approximately at noon. As fast as the spore suspension was consumed during the afternoon, the pans were replenished with additional quantities. At twilight the pans were filled for the last time. The spore suspension was absorbed, as were also dye solutions (discussed in a later section) at the rate of 1 to 2 liters per hour between the hours of noon and 6 p. m. Spores were not recovered in the early (September) trials. Discoloration of the vessels and subsequent recovery of the fungus by culture demonstrated, however, that spores had been distributed to distances of 10 and 19 feet below the point of injection in these trees (24 and 20 of table 6). Spores were recovered in subsequent trials. They were observed under the microscope in the displaced sap in a number of instances. Twenty-four hours after injection they were recovered 13 and 17 feet below injections made at 24 and 25 feet,

TABLE 6.—*Distribution of spores of Ceratostomella ulmi below points of injection in the tops of American elms, 1936*

Tree No.	Injection date	Seasonal condition of trees	Time between injection and sectioning [1]	Tree dimensions	
				Diameter breast high	Height [2]
			Days	*Inches*	*Feet*
17	May 1	Leaves unfurling	3	4	20
18	do	do	3	4	20
19	do	do	3	4	20
			Hours		
20	Sept. 17	Prior to autumn leaf fall	17	9	25
21	Oct. 5	do	20	6	21
22	do	do	24	7	25
23	Oct. 13	do	24	8	24
24	Sept. 23	do	42	9	29
25	Oct. 7	do	46	7	27
26	Oct. 13	do	46	8	25
			Days		
27	Sept. 23	do	5	6	24
28	do	do	5	6	24
29	Oct. 5	do	14	9	34
30	Sept. 23	do	21	9	34
			Hours		
10	Nov. 11	Dormant	1	7	13
11	do	do	1	6	13
12	Nov. 6	do	3	7	13
13	Nov. 9	do	3	6	23
14	Nov. 12	do	27	7	23
			Days		
15	Dec. 2	do	4	8	22
16	do	do	14	7	24

Tree No.	*C. ulmi* isolated from sap displaced at indicated distance (feet) below injection point [3]							Lower limit of vascular discoloration	Maximum distance below injection point at which *C. ulmi* was isolated from discolored vessels
	1–3	4–7	7–10	10–13	13–17	17–21	21–25		
								Feet	*Feet*
17	−	−	−					2	2
18	−	−	−					2	2
19	−	−	−					1	1
20		−	−	−	−			23	19
21		+	+	−	−			21	19
22		+	+	+	+	−	−	25	21
23		+	+*	+	+	−	−	23	17
24		−	−	−	−	−		18	10
25		+*	+	+*	+	+	+*	27	27
26		+	+	+	−	+	−	25	21
27								24	20
28								20	20
29								34	34
30								34	34
	1–4		4–8		8–12		12–16		
10	+		−		−		−	[4]	4
11	+		−		−		−	[4]	5
12	+		+		+		−	[4]	12
13	+		+		−		−	[4]	8
14	+		+		−		−	[4]	8
15	+		+		+		−	[4]	12
16	+		+		−		−	[4]	8

[1] The interval between the beginning of the injection and the time at which the tree was felled and sectioned.

[2] All vessels of the several outer rings were injected with spore suspension at the height above the stump indicated. The diameters of the stems at the point of injection ranged from 2 to 3 inches.

[3] + indicates that *C. ulmi* was isolated; −, that it was not. Typical yeastlike spores (fig. 3) of *C. ulmi* were observed in the sap samples taken from the injected tree sections designated by an asterisk (*).

[4] No discoloration developed.

respectively, above the stump; 46 hours after injection they were observed and identified at 25 feet below an injection made 27 feet above the stump.

Several weeks after the trees had been cut down, vascular discoloration was found to be abundant within several feet of the injection point; this discoloration became progressively less with increasing distance below the injection point; in most instances it finally failed as a few scattered streaks or a single one at the limits indicated (table 6). On the stem of tree 25, for example, several hundred streaks (dots in the outer spring vessel ring in transverse sections) were present 13 feet below the injection point; 54 were present at 20 feet, 33 at 24 feet, and 5 at the stump, or 27 feet below the injection point. *Ceratostomella ulmi* was consistently isolated from all 5 streaks in the stump and from streaks at other points from which recovery was attempted in this tree. In the stem of tree 22, 20 streaks developed at 17 feet below the injection point, 7 at 20 feet, and only 1 at 25 feet below. *C. ulmi* was isolated at all points from which recovery was attempted, except at 24 feet.

Several trees that were top-injected were not examined or cut down until 5 to 21 days after injection. Discoloration was detected in the outermost spring vessels of the stem of tree 28, 5 days after injection. It was steel blue in color and of rather low intensity. Within 10 feet of the injection point it was much more conspicuous by virtue of the fact that many vessels were discolored. About a month later a final examination revealed the usual deep-brown discoloration in the vessels through which spores of *Ceratostomella ulmi* had been distributed. Two streaks extended to 20 feet (4 feet above the stump) in tree 28 (table 6). *C. ulmi* was recovered from both streaks within an inch of their termini. Two typical streaks, one involving four or five vessels, extended into the stump 24 feet below the injection point in tree 27. *C. ulmi* was recovered from both at 20 feet, but not at 24 feet, below the injection point. Six streaks of discoloration, each embodying one to several vessels, were found in the stump 34 feet below the injection point in tree 29, which was cut down 2 weeks after injection. *C. ulmi* was recovered from each of the four streaks cultured. Trees cut at 3 and 4 weeks, respectively, after injection yielded like results (table 6).

Spores of *Ceratostomella ulmi* were injected at the top of the bole in several small and large trees during the early half of the leafy season. The spores were injected by the pan and chisel-cut procedure for 15 minutes to 1 hour. The trees were then felled. The large trees were cut into 10- to 20-foot logs; the small ones were not sectioned. All were defoliated within 2 hours after felling. The tops of the large trees were not cut off until after felling.

One month after injection, discoloration induced by the spores extended in the outer ring of vessels to the stump (fig. 5, *A*) in all trees injected directly with spore suspension (table 7). In trees 1, 2, and 3, respectively, 39, 5, and 5 vessels of the new ring were discolored by the spores at the stump, or roughly 7 feet below the injection points in the smaller trees. In the 2 tall trees injected directly with spore suspension, 15 and 20 of these vessels were discolored at the stump, 40 and 41 feet, respectively, below the injection points. Only 1 streak of discoloration was found at the lower limits of spore distribution 32 and 32.5 feet, respectively, below the point of injection in the 2 tall trees that were injected for 1 hour with sterile water prior to

spore injection. Each spot or streak of discoloration in the ring was interpreted as originating in 1 vessel. Several vessels may have been involved, however. *Ceratostomella ulmi* was recovered at representative points from these discolored vessels in all cases.

TABLE 7.—*Distribution of Ceratostomella ulmi from points at which spore suspension was injected into elms near the top of the main stem*

Tree No.	Injection date	Tree dimensions			Schedule of operations			Liquid absorbed	
		Diameter breast high	Height	Diameter at injection point	Water injected	Spore suspension injected	Tree felled	Water	Spore suspension
	1937	*Inches*	*Feet*	*Inches*	*Clock time*	*Clock time*	*Clock time*	*Liters*	*Liters*
85[1]	July 23	9	61.0	4.5	----------	2:12 p. m.	2:27 p. m.	----------	3
86	---do----	12	67.0	5.0	----------	10:50 a. m.	11:10 a. m.	----------	6
	1938								
41	June 28	10	57.0	3.3	2:25 p. m.	3:25 p. m.	4:25 p. m.	8	6
42	---do----	12	62.2	3.5	10:45 a. m.	11:45 a. m.	12:45 p. m.	16	13
1	June 2	1.6	13.5	1.0	----------	10:30 a. m.	10:50 a. m.	----------	.3
2[2]	---do----	1.2	12.0	.6	----------	10:40 a. m.	11:00 a. m.	----------	.3
3	---do----	1.3	12.4	.7	----------	11:10 a. m.	11:30 a. m.	----------	.3

Tree No.	Stem below points of injection										Stem above point of injection				
	Total length	Vessels discolored at distances indicated (feet) below injection point[3]								Levels below injection point at which *C. ulmi* was isolated	Total length	Terminus of discoloration			Maximum height above injection point at which *C. ulmi* was isolated
		5	10	15	20	25	30	35	40			Height above injection point	Stem diameter	Stem age[4]	
	Feet	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*No.*	*No.*	*No.*	*No.*	*Feet*	*Feet*	*Feet*	*Inches*	*Years*	*Feet*
85[1]	40.0	75	50	33	25	77	48	18	15	30,40	21.0	17.0	1	----------	17.0
			No.	*No.*	*No.*										
86	41.0	50	131	98	27	----	22	20	20	31,41	26.0	23.0	¾	----------	23.0
			Pct.	*Pct.*	*Pct.*										
41	38.5	75	50	----	25	5	3	0	0	24,32	18.5	----------	----------	----------	----------
42	41.0	95	90	----	50	----	3	0	0	20,25	21.2	20.5	5/16	3	20.5
		No.	*No.*												
1	8.0	35	[4] 39	----	----	----	----	----	----	6,8	5.5	4.8	3/16	3	4.1
2[2]	6.7	13	[5] 5	----	----	----	----	----	----	4,6.7	5.3	4.5	5/16	3	4.0
3	6.7	12	[5] 5	----	----	----	----	----	----	4,6.7	5.7	4.6	5/16	3	4.6

[1] *Ulmus fulva;* all others, *U. americana.*
[2] See fig. 5, *A.*
[3] Estimates of percentage of total vessels discolored in the outer ring are given where accurate determination was difficult as a result of fusion of many spots of color into a solid band (fig. 8). Each spot was considered as one discolored vessel; actually it usually involved several.
[4] Determined from terminal growth scars. In each case listed the age of the branch at the point indicated was equal to the number of vessel rings present plus 2.
[5] Number of spots of dicoloration in outer ring at stump or base of tree.

INJECTION OF A FEW DROPS OF SPORE SUSPENSION INTO TERMINAL BRANCHES

Relatively small quantities of spores were introduced into the vessels of terminal branches in injections made through chisel cuts during the dormant season of early spring and in midsummer. The injections were made with a ¼-inch chisel, 8.6 to 29 feet above the stumps, in terminal branches one-half to 1 inch in diameter. Certain other trees not recorded in the tables and injected in early spring were felled 6 weeks after injection; the distribution of spores was traced at once from discolorations that had been induced in the injected vessels

by t e spores introduced. The trees injected in midsummer were felled 1, 5, and 14 days after injection and were immediately defoliated. One month later the trees were examined and the distribution of spores was traced by the discoloration procedure.

The spores injected in this manner April 1, 1937, into 12 dormant trees approximately 40 feet in height did not descend more than 19 inches in 6 weeks. When the trees were felled May 11, the maximum extension of discoloration in the outermost ring of large vessels injected was 8 inches above and 19 inches below the injection point. The average distribution in these vessels below the injection point was 5.4 inches; the average distribution above, 1.4 inches. *Ceratostomella ulmi* was isolated from the discolored vessels of the 1936 ring at representative points above and below the injection points.

TABLE 8.—*Distribution of Ceratostomella ulmi 24 hours after spore injections made in chisel cuts July 29, 1937, into the terminal branches of American elms*

Tree and branch No.	Tree dimensions		Injection point		Stem below injection point							
	Diameter breast high	Height	Stem diameter	Stem age	Total length	Vessels discolored [1] at indicated distance (feet)						
						1	2	3	6	9	12	16
	Inches	*Feet*	*Inch*	*Years*	*Feet*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
9-1	3.8	27.5	9/16	5	24.0	16	12	9	5	6	2	1
13-1	2.0	18.5	1/2	5	13.2	16	8	7	1	0	----	----
14-1	2.8	21.8	1/2	5	17.3	1	1	0	----	----	----	----
14-2	----	----	1/2	6	17.7	4	3	1	0	----	----	----
15-1	3.3	20	1/2	5	14.5	4	3	1	0	----	----	----
15-2	----	----	5/8	5	8.6	16	13	9	1	0	----	----
16-1	3.0	18.3	7/16	5	14.8	13	9	7	3	0	----	----
16-2	----	----	7/16	5	14.8	5	4	5	5	2	0	----
17-1	2.5	22	1/2	4	18.0	2	2	1	0	----	----	----
17-2	----	----	1/2	4	18.0	8	8	8	5	2	1	0

Tree and branch No.	Stem above injection point								
	Total length	Vessels discolored [1] at indicated distance (inches)						Terminus of discoloration		
		6	12	18	24	36	48	Stem diameter	Stem age	Distance from tip of branch
	Inches	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Inch*	*Years*	*Inches*
9-1	43	12	3	1	0	----	----	1/8	3	25
13-1	64	19	11	----	6	1	0	1/4	2	28
14-1	53	3	1	0	----	----	----	1/4	3	41
14-2	48	3	3	2	1	0	----	5/16	3	24
15-1	66	13	----	----	5	----	1	1/4	2	22
15-2	71	----	8	----	2	----	1	1/4	2	23
16-1	41	----	5	----	1	0	----	1/4	2	29
16-2	41	3	1	1	0	----	----	1/4	3	23
17-1	48	1	1	0	----	----	----	----	----	36
17-2	42	13	8	2	2	0	----	1/4	2	28

[1] *C. ulmi* was consistently isolated at representative points from the discolored vessels in practically all trials made.

Spores injected in this manner at 37 points into the branches of 20 leafy trees 18 to 34 feet in height July 29, 1937, apparently descended as much as 16 feet in 24 hours and to the base of some trees in less than 5 days. A varying number of the vessels injected by the chisel injury were discolored in the 10 treated branches of 6 trees felled 24 hours after injection (table 8). Vessels not injected by the chisel injury were not discolored nor could *Ceratostomella ulmi* be isolated from them. *C. ulmi* was isolated with ease from the discolored vessels at representative points. The minimum distribution of spores as indicated by discoloration in the trees felled 24 hours after injection

was 1 foot, in tree 14–1 (table 8). The maximum distribution was 16 feet, in tree 9.

TABLE 9.—*Distribution of Ceratostomella ulmi 5 days after spore injections made in chisel cuts July 29, 1937, into the terminal branches of American elms*

Tree and branch No.	Tree dimension		Injection point		Stem below injection point								
	Diameter, breast high	Height	Stem diameter	Stem age	Total length	Vessels discolored [1] at indicated distance (feet)							
						1	3	6	9	12	15	18	29
	Inches	*Feet*	*Inch*	*Years*	*Feet*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
2–1	2.5	22.5	3/8	6	18.5	2	1	1	0				
2–2			1/2	5	18.0	2	0						
3–1	1.8	20.3	1/2	5	15.5	[2]±50	±50	22	18	18	16		
6–1	4	33.2	1/2	3	29.0	21	20	20	[3]				
6–3			1/2	4	29.0	16	15	12		36	41	23	6
6–2			1/2	6	24.0	20	16	22		20			
18–1	2.5	17.7	1/2	3	11.7	15	15	15	15	3			
18–2			1/2	3	11.2	±26	20						
18–3			3/4	3	12.5	±35	33	16					
19–1	3.5	22.3	5/8	4	18.3	14	14	8	50			35	
19–2			3/4	3	18.8	20	11	8					
19–3			1/2	3	18.8	±48	40	37					

Tree and branch No.	Stem above injection point						Terminus of discoloration		
	Total length	Vessels discolored [1] at indicated distance (inches)					Stem diameter	Stem age	Distance from tip of branch
		12	18	24	36	48			
	Inches	*No.*	*No.*	*No.*	*No.*	*No.*	*Inch*	*Years*	*Inches*
2–1									
2–2	54	[2]±30		24	10	0	1/8	1	10
3–1	58	28		19		2	1/8	1	7
6–1	42	5	4	1	0		1/8	1	15
6–3	50	5	4	1	0	0	1/4	2	18
6–2	55	11			6	1	1/8	2	7
18–1	48	±25		±20	3	0	3/16	1	8
18–2	68	8	5	5	4	0	3/16	2	27
18–3	46	±12	10	8	0		3/16	1	11
19–1									
19–2	66	16	16	16	10	3	3/16	2	22
19–3									

[1] *C. ulmi* was consistently isolated at representative points from the discolored vessels in practically all trials made.

[2] Fusion of the spots of discoloration made accurate determination of the number of vessels involved difficult.

[3] The branches fused into a single stem between the points indicated by the braces; for example, branches 1 and 3 of tree 6 fused 7 feet below the points of injection.

Discoloration was present to the ground line in 4 of the 5 trees felled 5 days after injection. The fungus had gained access by this time to many vessels all around the stem, most of which had not been injected. An indeterminable number of vessels were discolored 1 foot below the injection points in 4 of the 12 leader branches injected in these trees. The maximum number of vessels discolored at the ground level was 35, in tree 19; the maximum distance of spore distribution, as indicated by discoloration, was 29 feet, or to the stump, in the tallest tree examined at this time, tree 6 (table 9). Only two vessels were discolored below the injection point in each of two leaders injected in tree 2. Six feet was the maximum downward distribution of discoloration in this 22.5-foot tree injected 18 feet above the stump.

Two weeks after injection, discoloration involved practically all vessels near the injection points in the trees left to stand until this time; it was widespread in most branches of the trees and had reached the soil level in six of seven trees examined in detail.

Discoloration developed slowly in side branches that came off the injected axis below the injection point in these trees. Only one of the branches that came off the injected axis below the injection point developed any discoloration in 30 trees felled and defoliated within a few hours or up to 1 day after injection. There was extensive discoloration in some of the lower side branches in trees felled and defoliated 5 days after injection. Of the branches below the injection point, those nearest the injection point developed discoloration first. The extent of discoloration in some of these large branches was as great as in those above the injection point 5 days after injection. It extended only a few feet into many at this time, however. Two weeks after injection there were still numerous lower lateral branches free from discoloration.

INJECTION OF SPORE SUSPENSION INTO TERMINAL SHOOT GROWTH OF CURRENT SEASON

Spores of *Ceratostomella ulmi* were injected into the terminal shoot growth of the current season at the top of trees 10 to 15 feet in height. The injections were made by excising these shoots 2 to 4 inches from their ends after bending them down and submerging the parts in a concentrated spore suspension in a pail. The cut ends were held under the suspension for 1 hour. The excised tips were discarded. The trees were then cut and defoliated and left lying in the shade of a dense thicket for a month before inspection for discoloration was made.

TABLE 10.—*Distribution of Ceratostomella ulmi below points at which spore suspension was injected* [1] *for 1 hour into apical stem growth of the current season*

Tree No.	Shoot No.	Maximum distribution of spores below injection point determined by—		Terminal stem growth of the current season	
		Vascular discoloration induced	Isolation of *C. ulmi* [2]	Total length remaining after injection	Length not invaded
		Inches	*Inches*	*Inches*	*Inches*
51	1	3.5	3.5	12.0	8.5
	2	6.0	6.0	12.5	6.5
	3	0	0	4.0	4.0
52	1	6.0	6.0	17.0	15.0
	2	5.0	3.0	18.0	13.0
	3	3.0	3.0	19.0	16.0
	4	6.0	6.0	19.0	13.0
53	1	3.0	3.0	20.0	17.0
	2	3.0	3.0	19.0	16.0
	3	6.0	6.0	22.0	16.0
	4	3.0	3.0	19.0	16.0
	5	3.0	3.0	21.0	18.0
54	1	4.5	[3] 3.0	8.0	3.5
	2	6.0	5.3	18.0	12.7
	3	2.0	2.0	13.5	11.5
	4	2.0	.5	14.5	12.5
55	1	2.5	2.5	6.0	3.5
	2	6.0	4.0	17.0	11.0
	3	5.0	1.5	17.5	12.5
	4	4.0	3.0	14.8	10.8

[1] American elms injected Aug. 22, 1938, by excision under a suspension of *C. ulmi* spores. The point at which the stems were cut was 2 to 4 inches from their ends.

[2] 3 weeks after injection every inch of stem bearing vascular discoloration was cultured in trees 51, 52, and 53. The injected shoots were also cultured at 6, 12, 18, and 24 inches below the point of injection.

[3] Isolations from injected shoots of trees 54 and 55 were not made until February 1939.

Discoloration did not extend more than a few inches below such injection points in terminal stem growth of the current season, nor did it reach older wood in any of the terminal shoots injected. The diameters of the portions of the shoots invaded by *Ceratostomella ulmi* ranged from one-sixteenth to one-eighth of an inch. The organism injected was isolated in most cases to the limits of the discoloration recorded in early trials but not below the limits of discoloration (table 10). The downward distribution of the fungus in current season's stem growth was thus in striking contrast to that found for spores injected into the top of the bole or major branches of the elm.[7]

DISTRIBUTION OF SPORES OF CERATOSTOMELLA ULMI IN TOP-INOCULATED ELMS

Under natural conditions, spores of *Ceratostomella ulmi* probably are deposited in injured tissues rather than injected into the sap stream. To gain some concept of the rapidity of invasion of the vascular system under more natural conditions, a series of trees were top-inoculated and the results compared with those from trees that had been top-injected.

Inoculations were made into the tops of 27- to 49.5-foot trees July 16, 1937. The inoculations were made into the leader branch or

FIGURE 9.—Diagrammatic sketch of initial vascular invasion by *Ceratostomella ulmi* from a chisel-cut inoculation on a leafy elm branch. Streaks of discoloration from which *C. ulmi* was isolated appeared in line with the edges of the chisel cut 1 week after inoculation in July. The streaks were caused by invasion of the fungus in the functional vessels nearest the inoculated tissue.

branches within 5 to 15 feet of their ends. The diameters of the branches at the inoculation points ranged from 0.5 to 1.75 inches. The inoculated terminal branches or the entire trees were cut off 7, 19, and 116 days respectively, after inoculation. The branches removed after 7 and 19 days, respectively, were stripped of leaves, left in the shade for 2 or more weeks, and then examined for spore distribution by the discoloration procedure. The others were examined at once.

Seven days after inoculation of five branches treated in this manner, four showed no discoloration above or below the injury. In the fifth branch, streaks of discoloration in line with the edges of the chisel injury extended 14 inches above the injury and 10 inches below (fig. 9). The streaks were discolored vessels of the current season's growth sheath. There was but one streak on either side of the injury. The streaks in each instance involved several vessels (fig. 4, *B*).

The extent of vascular invasion 19 days after inoculation in 12 branches examined at this period is shown in table 11. Typical wilt symptoms of Dutch elm disease were shown by only 4 of these 12 branches at this time. Vascular discoloration extended into terminal shoot growth of the current season in all 4. Vascular discoloration was extensive above the inoculation points in 7 of the branches examined; it was limited in the other 5, however, to within 1 inch of the

[7] The results of Radulescu's (*89*) leaf-injection experiments, described in the literature review of this paper, are in decided contrast to the results obtained in these experiments.

inoculation points. Below the inoculation points, vascular discoloration extended on an average to roughly but two-thirds of the distance to which it extended above the inoculation points. Representative areas of the discolored tissue were cultured at the time of observation and *Ceratostomella ulmi* was consistently recovered from them.

TABLE 11.—*Distribution of Ceratostomella ulmi from inoculation points* [1] *in termina branches of American elms 19 days after inoculation*

Tree No.	Branch		Tree height	Wilt symptoms [2] Aug. 4	Stem below inoculation point			Stem above inoculation point			
									Terminus of discoloration		
	No.	Diameter at inoculation point			Total length	Lower limit of vessel discoloration	Vessels discolored [3]	Total length	Height above inoculation point	Distance below end of branch	Stem age
		Inches	*Feet*		*Feet*	*Feet*	*Number*	*Feet*	*Feet*	*Inches*	*Years*
12	3	0.50	30.4	−	22.0	0	0	6.1	0	84	9
	4	.50		−	20.0	.25	[4] 1	6.5	.6	96	9
	5	.75		−	23.0	.9	[4] 1	7.4	.7	70	7
	6	.63		+	22.0	[5] 6.0	----------	7.6	7.5	3	1
22	1	1.3	49.5	+	34.1	5.0	----------	11.1	10.2	10	1
	2	1.75		−	34.5	8.7	[4] 6	15.0	12.5	30	5
	3	1.5		+	34.3	8.7	----------	15.0	14.8	4	1
	4	1.25		+	33.7	4.8	----------	10.6	10.2	4	1
23	1	.75	40.2	−	30.0	0	0	10.2	.1	121	9
	2	1.20		−	26.5	3.8	3	13.5	1.0	150	10
25	2	1.0	43.0	−	31.7	1.2	[4] 2	8.9	5.8	37	4
	3	1.1		−	27.	[5] 8.7	[4] 7	8.5	6.0	30	4

[1] Spores of *C. ulmi* were placed on the surfaces of chisel cuts made in the wood, July 16, 1937.
[2] Plus sign (+) indicates presence; minus sign (−), absence of symptoms.
[3] *C. ulmi* was recovered by isolation at representative points from the vascular discoloration recorded in this table.
[4] The discolored vessels were in line with the edges of the chisel inoculation cuts as shown in figure 9.
[5] Vascular discoloration extended into the bole below the point at which this branch was cut from the tree. No observations were made below this point at this time.

Approximately 4 months after inoculation, all the remaining inoculated trees were felled, examined for vascular discoloration, and destroyed. Data for seven trees in which the extent of invasion above and below the inoculation point was traced in detail are shown in table 12. Foliar symptoms of disease had appeared only on the three trees in which vascular discoloration had reached the terminal shoot growth of the current season and in which nearly all vessels of the outermost ring in the upper half of the bole were discolored. Vascular discoloration was present in the stump, however, in only one of these three trees. In the other four trees it was limited to a few vessels in line with the edges of the chisel-cut inoculation injury.

Eleven of the 25 trees top-inoculated displayed symptoms of disease by September 15, 1937. Three of these are shown in table 12. Vascular invasion had reached the stump in only 4 of these trees by November 9, 1937. The 13 other trees showed no symptoms at any time, yet in 3 of these trees vascular discoloration extended into the stump by November 9. Data for one of these trees (tree 25) are shown in the table. *Ceratostomella ulmi* was found associated with the vascular discoloration recorded in these trees at practically all representative points cultured. It is apparent from these experiments that although the fungus may be distributed from top to bottom and throughout most branches of a tree within a few hours after injection, its distribution subsequent to its establishment in an injury is not predictable.

TABLE 12.—*Distribution of Ceratostomella ulmi from inoculation points*[1] *in terminal branches of American elms, 116 days after inoculation*

Tree No.	Branch		Tree height	Wilt symptoms[2]		Stem below inoculation point			Stem above inoculation point			
										Terminus of discoloration		
	No.	Diameter at inoculation point		Aug. 4	Sept. 15	Total length	Lower limit of vessel discolored	Vessels discolored[3]	Total length	Height above inoculation point	Distance from end of branch	Stem age
		Inches	*Feet*			*Feet*	*Feet*	*Number*	*Feet*	*Feet*	*Inches*	*Years*
6	1	0.80	48.0	−	+	34	26.0	[4] ∞	14.0	13.7	5	1
19	1	1.25	37.3	+	+	29	22.0	∞	8.3	8.0	5	1
20	1	1.25	42.0	−	−	28	12.0	[5] 4	14.0	7.0	24	4
23	1	1.50	40.2	−	−	27	8.0	[5] 4	13.0	3.0	120	9
24	1	.75	27.0	+	+	19	19.0	∞	8.0	7.8	2	1
25	1	1.50	43.0	−	−	30	1.5	[5] 3	13.0	5.0	96	10
25	3	1.1	43.0	−	−	27	27.0	[5] 6	[6] 8.5	6.0	20	4

[1] Spores of *C. ulmi* were placed on the surface of chisel cuts made in the wood July 16, 1937.
[2] + indicates presence, − absence of symptoms.
[3] *C. ulmi* was recovered by isolation at representative points from the vascular discoloration recorded in this table.
[4] Nearly all vessels of the outer ring were discolored in the upper part of the bole in these trees.
[5] The discolored vessels were in line with the edges of the chisel inoculation cuts as shown in figure 9.
[6] This branch was excised August 4, 1937.

PRESENCE OF SPORES OF CERATOSTOMELLA ULMI IN SAP DISPLACED FROM ELMS AFFECTED BY DUTCH ELM DISEASE

The rapid progress of *Ceratostomella ulmi* in the vessels of trees into which no spores were injected is evidence that the fungus produces spores or free drifting mycelial fragments in the vessels to which it gains access by tissue penetration in the vicinity of the injuries involved in the inoculation procedure. Spores have been seen in histological preparations of the discolored vessels of stems from naturally diseased and from inoculated trees (*4*). Verification of the presence of spores in the vessels of stems of diseased trees at various times during the leafy season of 1936 was obtained experimentally by the following procedure.

TABLE 13.—*Presence of Ceratostomella ulmi spores in sap displaced from American elms affected by the Dutch elm disease, 1936*

Date	Tree designation	Height at which sap was displaced	Yeastlike spores in centrifuged sap	*C. ulmi* colonies identified in each plate[1]	Date	Tree designation	Height at which sap was displaced	Yeastlike spores in centrifuged sap	*C. ulmi* colonies identified in each plate[1]
		Feet		*Number*			*Feet*		*Number*
June 15	9B	2–4	−	3, 3, 3	Aug. 17	K_1	22–26	+	0, 0, 0, 0
Do	9B	16–20	[2] −	7, 14, 8	Do	K_3	36–40	+	0, 0, 0, 0
July 1	21B	2–4	+	.....	Aug. 18	1071B	12–16	+	0, 0, 0, 0
Do	21B	12–16	+	.....	Do	16B	12–16	+	3, 2, 4, 3
July 3	19B	4–7	+	0, 0, 0, 0	Do	1072B	8–12	−	0, 0, 0, 0
Do	19B	7–11	+	0, 0, 0, 0	Do	K_2	36–40	+	10, 23, 5, 9
Do	19B	12–15	+	12, 3, 2, 0					

[1] 1 cc. of the optimum dilution of the sap sample was flooded over each of 3 or 4, 9-cm. Petri dishes containing solidified acid malt agar.
[2] − signifies absence; +, presence of yeastlike spores of *C. ulmi*.

Sap was displaced from 2- to 4-foot sections of the stem of diseased trees at various periods of the year. Naturally diseased (K) and "inoculated" trees (B) were used. The work was done only on those trees that displayed characteristic foliar symptoms of disease during the leafy season of 1936. All organisms contained in the displaced sap were concentrated into a 0.2-cc. volume by centrifuging. Yeastlike spores (fig. 3), typical of those produced by *Ceratostomella ulmi* in liquid culture, were seen in most samples (table 13). The 0.2-cc.

concentrates were resuspended as described for this technique earlier, and the suspensions were cultured. Colonies of *C. ulmi* were identified in cultures of the sap displaced from four of nine trees studied.

DISTRIBUTION OF DYES SUBSEQUENT TO INJECTION INTO ELMS AT VARIOUS SEASONS

Dyes were injected into elms at various seasons of the year. Their distribution served as an index of the movement of the sap stream from injection points to other parts of the tree. The pan and chisel-cut injection procedure was used. The injection periods ranged from 1 to 84 hours. The trees were felled at the end of the injection period, at which time the locus and extent of dye distribution in trunk and crown of the injected trees were traced by inspection for dye in cross sections of the stem. Acid fuchsin and light green were the dyes used. Trees in the same stands and similar to those used in the spore-distribution experiments were employed. A record of the limits of distribution of these dyes in trees base-injected at various seasons of the year appears in table 14.

The dyes were observed in the lumina of many of the tracheae of small diameter of all growth sheaths severed by the injection chisel. They also appeared in the walls of contiguous cells. The dyes rose for several feet in this system of vessels in trees injected during the leafy season. The vessels of large diameter of the new ring, however, were the channels whereby dye was distributed throughout the crown in the leafy season. The dyes were not observed in tracheae of large diameter of any ring in those trees injected during the spring flowering period or during the dormant season.

TABLE 14.—*Distribution of water-soluble dyes injected stump high into American elms, 1936–37*

Tree No.	Date	Seasonal condition of tree	Injection time	Tree dimensions		Maximum height reached by dye
				Diameter breast high	Height [1]	
	1936		*Hours*	*Inches*	*Feet*	*Feet*
1	Apr. 13	Flowering, no leaves	72	3	20	5.2
2	do	do	72	4	29	7.8
	1937					
3	Apr. 26	do	3	5	59	4.2
4	do	do	3	7	59	3.3
45	May 11	New leaves expanding	2	6	40	22.0
47	do	do	2	4	35	20.0
48	do	do	2	4	35	25.0
5	July 19	Full leaf	[2] 18	9	55	55.0
6	do	do	[2] 18	9	55	53.0
	1936					
7	Sept. 10	do	3	4	31	30.0
8	do	do	3	4	27	26.3
9	Sept. 24	do	3	3	25	24.6
10	do	do	18	5	35	35.0
11	Nov. 3	Leafless, dormant	3	5	34	6.0
12	do	do	3	4	22	6.3
13	do	do	3	4	30	4.2
14	Dec. 3	do	84	8	48	21.0
15	do	do	84	10	57	21.0

[1] Measured from the point of injection.
[2] Chemically girdled trees in which the cambium was killed, during the preceding dormant period, over a 3- to 10-foot length of trunk at the base of the tree.

At the season of the year when the new shoots were rapidly elongating and the new leaves expanding, and at a time when the vessels of the new ring were not yet mature at the base of the trees, the dye rose 10 to 15 feet in the small tracheae of the growth sheaths penetrated by the chisel used in making the injections. It was distributed farthest, however, in the new tracheae of large diameter of the new ring, which were present in the upper half of the bole and crown at this time although not yet mature in the lower part of the bole. When trees were injected during the leafy season, the dyes were observed in the walls of the tracheae of the outer ring of large vessels and in contiguous cells in the crown. These vessels were colored by the dyes throughout the crowns of the trees injected during the leafy season to the heights indicated in the table. When injection at this season was continued for 18 hours all leaves were stained red by the acid fuchsin.

Dyes were injected into the girdled area of leafy trees that had been banded with copper sulfate during the preceding dormant season, by a procedure similar to that described by Bedard (*6*), Lantz,[8] and Liming.[9] The cambium in such trees had been killed over a distance of 3 to 10 feet above the girdle all around the trunk. The dyes after injection into these trees overnight were observed in the lumina of many tracheae of small diameter scattered through the wood of all the growth sheaths penetrated by the injection chisel up to 35 feet above the injection point. Although such dye-conducting vessels were generally scattered at random through all sap rings penetrated by the injection chisel (fig. 4, *C*), there was a pronounced tendency toward dye conduction in rings of vessels. These rings of color seemed to coincide with the rings of large spring vessels. Close inspection revealed, however, that dye was in the lumina of only the small vessels of the late summer wood contiguous to these rings. There was no convincing evidence that dye was distributed in the vessels of any of the rings of large tracheae formed prior to the season of injection. Dye was widely distributed in the new ring of large spring vessels formed above the girdled area. It was observed in this system of vessels even in the small branchlets at the top of the two trees studied.

Dyes were injected into the top of the boles of a few trees during the leafy and dormant seasons. The path of distribution was the same as for base-injected trees. The distance to which the dyes descended below the injection point varied. After 72 hours of injection in November in one tree at 30 feet above the ground, light green was detected in the tissue around the outer ring of large vessels at 25 and 27 feet below the injection point. Three hours after injection in two leafy trees at 20 feet above the stump October 1, acid fuchsin was seen in a few vessels 6 inches above the stump in one of them. In the other it descended only 12 feet 10 inches below the injection point. It descended in the outer ring of vessels in these trees to branches at various distances below the injection point, then moved out toward the leaves in these branches, in some cases reaching the leaves. Light green followed a similar course in the trees injected after leaf fall, but did not reach the distal portion of the lateral branches.

[8] LANTZ, A. E. AN EFFICIENT METHOD FOR INTRODUCING LIQUID CHEMICALS INTO LIVING TREES. U. S. Dept. Agr., Bur. Ent. and Plant Quar. E 434, 4 pp., illus. 1938. [Mimeographed.]

[9] See footnote 6.

RATE OF DISTRIBUTION OF HIGHLY COLORED SUSPENSOIDS AND STAINED YEAST CELLS INJECTED INTO ELMS

Highly colored suspensoids and stained yeast cells were injected into elms (*Ulmus americana* and *U. fulva*) at various seasons of the year. The pan and chisel-cut injection procedure was used. The distribution of stained spores or suspensoids was observed in situ in the new ring of vessels at the season of the year when little wood had as yet been laid down over the new vessel ring. In such cases, the bark was removed immediately before injection. The highly colored particles injected moved through the vessels of the new ring for varying distances and produced in these vessels the same spotted or discontinuous streak pattern that the various elm wilt fungi generally induce. These suspensoids during the months of June and July commonly ascended the first 5 feet in 8 to 15 seconds and reached their maximum upward distribution of 8 to 20 feet in 2 to 10 minutes. They descended from injection points in the bole at the base of the crown with like speed. Two minutes after injection, June 24, 1937, india ink had descended to 14.5, 18.3, and 19 feet in three trials from injections made 21 feet above the stump. It descended 36 feet in 6 minutes from an injection made June 25, 1937, 38 feet above the stump. All this distribution occurred in the outer or new ring of vessels. The carbon particles of india ink, suitably diluted, Turnbull's blue, iron oxychloride, and beer yeast cells stained with Heidenhain's iron-alum haematoxylin were not distributed in any of the large spring-formed vessels of inner rings but were distributed to a distance of less than 16 inches in the vessels of the summer wood of all growth sheaths injected. During the nonleafy period of the year, distribution of these suspensoids was limited to the vessels of the summer wood but did not exceed 16 inches above or below injection points made in the boles of trees 6 to 12 inches in diameter breast high.

DISCUSSION

RELATION OF DRIFTING SPORES TO EXTENSIVE RAPID FUNGUS INVASION OF THE TRACHEAL SYSTEM IN THE ELM

The experimental evidence presented demonstrates that rapid and extensive spread of *Ceratostomella ulmi* in the American elm is due to the distribution of its spores, drifting in the sap stream. Limited evidence presented indicates that the two other fungi known to induce vascular wilt diseases of the elm, namely, *Verticillium dahliae* and *Dothiorella* (*Cephalosporium*) *ulmi* are distributed in the same way in the elm. Experiments [10] with living cells of the beer yeast *Saccharomyces cerevisiae* Hansen have demonstrated that these cells may be distributed with like speed and essentially as far in the vascular system of the American and the slippery or red elm. From this it seems probable that other organisms would be distributed in the same manner, if their spores were produced in the functional vessels in question.

Spores of *Ceratostomella ulmi* have been seen and identified in sap collected from all levels of the boles of injected trees, up to 35 feet in height, within a few hours after injection at one point. They have

[10] See footnote 4.

been observed in sap taken from the boles of diseased trees months after initial infection. They have been seen in histological sections in vessels of small branches of the crowns and of the boles of inoculated and naturally diseased trees (*4*).

The presence of any of these organisms in functional vessels has been found in these studies to be consistently associated with discoloration of the vessels so occupied. This discoloration has been produced by all three fungi to the limits of their distribution but not elsewhere. It has been traced throughout the crown and bole of trees that have been felled, sectioned into 3- or 4-foot lengths, and defoliated within a few hours after spore injection, and in most cases the fungus injected has been demonstrated to be present in the discolored tissues but not elsewhere.

Invasion of elm tissue by mycelial growth of *Ceratostomella ulmi* is exceedingly slow, comparatively. Within the several hours' time in which the spread of *C. ulmi* spores occurred in the vessels of certain trees injected with spore suspensions of *C. ulmi*, spores in uninjected portions of these suspensions held in Erlenmeyer flasks in the laboratory and on the ground in the vicinity of the injected trees were found to reproduce almost exclusively by yeastlike multiplication. Spores from suspensions that had been thickly sown on nutrient agar media and had been held under the same conditions also reproduced practically exclusively by yeastlike multiplication. The maximum distance to which *C. ulmi* can grow along the lumina of vessels under optimum conditions is not precisely known. Initial trials,[11] however, have demonstrated a growth of 4 cm. parallel to the vertical axis of stems when they were held in a moist chamber for 2 months. It is obvious, therefore, that distribution by growth to the limits found in these experiments would be impossible within the time indicated.

These findings provide a new and fundamental concept of the mechanics of invasion in these and probably other vascular wilt diseases.

VESSEL LENGTH IN RELATION TO SPORE DISTRIBUTION

The distribution of spores or suspensoids in the elm is conditioned by the length of the tracheae into which they are introduced. De Bary (*5*) concluded that "As regards the absolute size of vessels, there is nothing to oppose the view that their length may equal that of the whole plant or at least may be very great." Strasburger (*34*), and more recently Priestley and his associates (*26*, *27*, *28*) have presented experimental evidence that supports this view as to the length of tracheae, at least in ring-porous dicotyledons. That there should be vessels open from base to terminal branches in the crown of elms regardless of the height of the tree was, therefore, to be expected. Extensive rapid distribution of spores throughout American and slippery elms occurs only in the new or outermost ring of vessels. The distribution of spores within a few hours to days after injection was found in this work to be limited to a few inches from points of injection in leafy American elms prior to the maturation of these vessels. It was likewise limited in girdled areas of leafy American elms in which no new ring of vessels was formed.

[11] See footnote 4.

ROLE OF SAP STREAM IN DISTRIBUTION OF SPORES

The role of the sap stream in the distribution of the wilt-inducing fungi studied is apparent from comparison of their spread from injection or inoculation points in dormant trees with that in leafy trees. When injected into dormant trees they were not spread more than a few inches in several weeks from points of introduction, whereas in the leafy season they were spread throughout the greater part of trunk and crown of small or large trees within a few hours. The speed of spore and water movement from injection points in leafy trees, judged from observation of dye and colored suspensoids in situ, is apparently a matter of several feet in a few seconds in either direction from injection points. Preston (*25*) reports initial rates of injections for dilute india ink of 1 m. per second in *Fraxinus americana* L. The distribution of spores of *Ceratostomella ulmi* above points of injection or inoculation in the elm is a function of the transpiration stream.

The reversal of the direction of this flow, as shown by the downward movement of dyes, suspensoids, and spores in these experiments, is similar to the reversal in the flow through the xylem reported more than two centuries ago by Hales (*19*) and in recent years by Arndt (*1*), Dixon (*14*), Dixon and Ball (*15*), and Yendo (*42*). The experiments of these investigators show that under the experimental conditions induced, tension created by the drawing off of water from transpiring leaves "determines a flow from any source wherever situated, and the continued transpiration from the leaves draws the supply through the plant along the channels of least resistance" (*14, pp. 61–62*). This loss of water by transpiration exceeds water intake, is cumulative, and induces "saturation deficits" in the tissue during the leafy seasons, according to Shull (*32*), and Smith, Dustman, and Shull (*33*). The downward translocation of liquids in the vascular system as in these experiments, as well as the initial rapid translocation above injection points, is thus a consequence of saturation deficits in the bole and roots of the elm, which would be relieved from any direction by a supply of free water, and, in Shull's words (*32*), "has nothing to do with the normal transpiration or translocation currents" in the plant. It would seem obvious, therefore, that this same rapid distribution of spores in either direction from points of injection in the bole of the elm would occur at any time during the leafy season, by night or day, in sunshine or rain. Experience has shown that this is essentially what happens.

INJECTION OF SPORES IN NATURE

The injection of spores into functional vessels of the elm apriori might seem to be a highly artificial procedure. However, any agency that severed these vessels would bring about the injection during the leafy season of such micro-organisms as happened to be in suspension in free liquid at the point of vessel rupture. In nature it would seem that injections of this type might be made by bark beetles. Since there is ample evidence that certain of these beetles carry spores of *Ceratostomella ulmi* externally and internally (*7, 16, 24, 32*), these spores, as well as other micro-organisms, probably are present in cell sap released by the burrowing of these insects. At the moment a functional vessel is severed, some of this liquid and any spores suspended therein would be drawn into the vessel.

Streaks of discoloration that extend from bark beetle galleries have been reported on various ring-porous trees (*20*). From such streaks on elm, *Ceratostomella ulmi* has been isolated by a number of investigators. Streaks of this kind were repeatedly seen on elms felled by the writer in early June and examined within 10 days after felling. Usually they extended for a distance of several feet above and below the point of vessel rupture. These streaks consisted of individual vessels or small groups of vessels of the new ring, which, as cultural study demonstrated, had been invaded by various fungi and bacteria. These organisms gained access to the vessels at the point ruptured by galleries of *Scolytus* or *Hylurgopinus*. Such a distribution appears to have been effected by the movement of sap or rain water in either direction from the point of rupture in these vessels and not by growth of the organism involved. India ink injected into the bole of recently felled elms was distributed in the same manner several feet above and below the point of vessel rupture or injection.

The bark beetles are the only demonstrated means, except root grafting, of transmitting *Ceratostomella ulmi* from diseased to healthy trees in nature (*12*, *17*, *23*, *30*). Injection of spores into functional vessels by these beetles probably occurs, although inoculation, i. e., the deposition of the fungus on the surface of injured tissue, from which it could grow into functional vessels, might be the more usual method.

DISTRIBUTION OF CERATOSTOMELLA ULMI FROM INOCULATION POINTS

The distribution of *Ceratostomella ulmi* from inoculation points occurs much more slowly than from injection points. Particularly is this true of distribution below inoculation points in the bole or crown of large trees. It results from several factors. (1) The fungus first has to gain access to functional vessels by mycelial growth from the inoculated tissue. (2) Spores must be produced in the invaded vessels. (3) The distribution of these spores below inoculation points probably depends either on reversals in the flow of sap in the invaded vessels or on precipitation of spores by gravity in invaded vessels wherein upward sap flow has been brought to a standstill by extensive tylose formation, which in most cases follows invasion by the fungus (*8*). Dr. A. L. Smith [12] found that some of the spores present in a spore suspension of *C. ulmi* fell through a still water column 5 feet in 30 minutes. (4) It would seem that invasion, production, release, and distribution of spores must occur in advance of occlusion of the areas of vessels occupied by tyloses.

VARIABILITY IN EXTENT OF VASCULAR INVASION IN ELMS FOLLOWING INOCULATION WITH THE WILT-INDUCING FUNGI

Finally it should be remembered that, although spores of the wilt-inducing fungi may be distributed throughout the greater part of the current season's growth sheath in even large trees within a few hours after such highly artificial injection procedures as are described in these experiments, invasion by these fungi under natural conditions is probably highly variable. This is suggested by the variable results that follow inoculations with these fungi. General and rapid invasion has practically always resulted, in the writer's experience, from chisel-cut injections with *Ceratostomella ulmi* spores, made during

[12] See footnote 4.

the month of June into the bases of American and slippery elms. No such invasion was found in dormant trees in these experiments. After the July top-inoculation experiments described in this paper, invasion of the vascular system of American elms was erratic, in most cases limited to the crown, and generally unpredictable. Smucker [13] found invasion to be limited to within a few centimeters of inoculations made with *C. ulmi* in the summer wood of crotches in the crowns of 4-foot trees treated in late July. Moreover, discoloration to the limits indicated was found in only 21 of the 50 cases studied. Invasion was likewise localized in 31 of 35 trees wherein the inoculation injury severed the functional ring of vessels in crotches during July. Invasion was also limited to within a few centimeters of similar inoculations in 31 of 40 trees wherein inoculation was made at the same time in the upper part of the trunks. Examination was made in the latter two groups 1 month after inoculation.

Variable distribution of *Ceratostomella ulmi* in elms in nature is evident from the reports of Ahrens, Shuttleworth, and McMaster,[13] and of True and Slowata (*35*). The first-named writers reported on 185 trees that were found to have recurrent symptoms of Dutch elm disease during the season following that in which the earliest ring vessels discolored, by the fungus were produced. They stated that vascular invasion during the season of initial infection was confined to one-fourth of the crown in 40 of these cases and that it was general throughout the entire tree in but 108 cases. Their study was based on discoloration in the penultimate ring of vessels from which *C. ulmi* was isolated. True and Slowata, reporting on a study of symptoms of Dutch elm disease in natural infections during the leafy season, stated that "infections unaccompanied by symptoms were mostly localized" in the large elms they studied.

Infections by *Cephalosporium* in most cases remain confined to a "limited portion only of the current season's growth" in infected twigs during the season of infection, according to Creager (*13*). Rapid invasion of the entire tree generally followed from the inoculations made at the base of the trunks of small trees by Goss and Frink (*18*).

Verticillium infections generally are believed to occur through the soil. Wollenweber (*40*), however, reported invasion of the vascular system as confined to within a few inches of points of inoculation in leafy trees in the autumn.

SUMMARY

METHODS

Spore suspensions of *Ceratostomella ulmi* were injected in relatively large volumes (0.1 to 89 liters) into the bases or tops of small and large elms during all seasons of the year. Several hours to 5 weeks after injection, determination of the limits and loci of distribution of the spores in these trees was made by one of two procedures. (1) Sap was displaced from representative 3- or 4-foot lengths into which the trees were cut immediately after completion of the injection period; the sap samples were examined for *C. ulmi* spores after concentration in a centrifuge; the spores observed were subsequently identified from the colony character and fructifications to which they gave rise when

[13] See footnote 4.

isolated on nutrient agars. Or (2) the trees were felled immediately at the close of the injection period, cut into 3- to 20-foot lengths at once, and then defoliated; the discoloration of the vessels through which spores had been distributed was traced several weeks after the injection; *C. ulmi* was isolated from the discolored vessels at representative points.

Small quantities of spores were also introduced in suspension through chisel-cut injections or were applied without free water to the surface of chisel-cut injuries at various points in the bole or crown of American elms up to 49 feet in height. Terminal shoots were injected by excision under spore suspension. Distribution of the fungus applied in these procedures was traced from the vascular discoloration induced and was confirmed by isolation of the organism.

RELATION OF SEASON TO DISTRIBUTION OF CERATOSTOMELLA ULMI IN THE ELM

Distribution of the fungus in the tree was profoundly influenced by (1) the season at which injection was made, (2) the locus of injection, and (3) the method of introduction into the tree.

During the early half of the leafy season *Ceratostomella ulmi*, injected in spore form in large volumes of suspension stump high, was recovered to within a few inches to feet of the terminal growth in small and large elms that had been felled and cut into 3- to 20-foot sections at the close of injection periods of 20 minutes to 48 hours. The average distribution was 10.7 feet in 9 small trees that averaged 12.9 feet in height, and 44.3 feet in 23 tall trees that averaged 49.6 feet in height. *Verticillium dahliae* and *Dothiorella* (*Cephalosporium*) *ulmi* were recovered within 1 to 11 feet of the tops of six 23- to 44-foot American elms 6 and 7 days, respectively, after injection of a few cubic centimeters of spore suspension at stump height. Injected in large volumes of spore suspension 40 or 41 feet above the stump at points where the stem was 4.5 or more inches in diameter, *C. ulmi* was recovered at stump height in trees felled at the close of 15- or 20-minute injection periods.

During the latter half of the leafy season, *Ceratostomella ulmi*, injected stump high in large volumes of spore suspension, could not be recovered at more than, roughly, one-half the height of twenty-five 13- to 60-foot trees, felled at the close of injection periods of 1 to 4.2 hours. Average maximum height at which the vessels were discolored by the spores was 10.8 feet in these trees, which averaged 29.7 feet in height. Injected 21 to 29 feet above the stump at this season in large volumes of spore suspension at points where the stem was 2 to 6 inches in diameter, *C. ulmi* was recovered at stump height in one of nine trees felled 17 hours to 5 days after inception of injection. The average maximum distance below the point of injection at which *C. ulmi* was recovered, however, was 19.3 feet in those trees wherein injection was made at an average height of 24.9 feet above the stump.

Spores of *Ceratostomella ulmi* were identified in sap displaced from various points in the boles or crowns of naturally diseased and inoculated trees that showed wilt symptoms in the months of June, July, and September, 1936.

During the nonleafy season, *Ceratostomella ulmi* could not be recovered at more than 24 inches above injection points in 12 trees

injected with large volumes of spore suspension and felled 1 hour to 28 days after the inception of injection. *C. ulmi* could not be recovered at more than 12 feet below similar injections made 13 to 24 feet above the stump in 10 trees injected 1 to many hours and felled 1 hour to 14 days after injection at this season.

RELATION OF METHOD AND LOCUS OF INTRODUCTION INTO THE TREE TO SUBSEQUENT DISTRIBUTION OF CERATOSTOMELLA ULMI IN THE ELM

Injected into terminal shoots for 1 hour, *Ceratostomella ulmi* could not be recovered at more than 6 inches below the point of injection in trees felled at the close of the injection period. Average distribution was 4 inches in 20 trials in August.

Injected into leader branches at points where the diameter ranged from three-eighths to five-eighths of an inch, *Ceratostomella ulmi* was recovered at 2 to 16 feet below injection points in trees felled 24 hours after injection in July. The average distribution below the injection point in 10 trials was 7 feet. The average height of injection was 16 feet.

Distribution of *Ceratostomella ulmi* from inoculation points was decidedly variable and took place more slowly than from injection points. One week after inoculation in July into top branches 0.5 of an inch to 1.75 inches in diameter, maximum distribution from inoculation points did not exceed 14 inches; 19 days after inoculation, maximum distribution was 14.8 feet; minimum, none; approximately 4 months after inoculation in 25 trees up to 49 feet in height, distribution varied from 1.5 feet from inoculation points to the entire branching structure and bole of the trees and was limited to a few vessels or was generally present in all large tracheae of the new annual ring in the crown structure. From comparable points at which a few drops of spore suspension were injected through chisel cuts in the nonleafy season, distribution at the end of 6 weeks did not exceed 19 inches above or below, and averaged 1.4 inches above, 5.4 inches below the cuts in 12 trees approximately 40 feet in height.

LOCUS AND RATE OF DISTRIBUTION OF SPORES AND OTHER COLLOIDS IN THE ELM

The tracheal system was the locus of spore distribution. Spores and suspensoids were distributed by the sap stream in the small vessels of the wood and in the large vessels of the outer annual ring. They were distributed through the smaller vessels of the wood of all growth sheaths severed by the injection chisel. The distribution in these vessels was never found to be greater than 18 inches above or below injections made at any season in trees felled within 24 hours after inception of injection. Distribution of spores beyond 18 inches was almost exclusively through the large vessels in the outermost or new ring.

Highly colored suspensoids and stained yeast cells were observed to move in the large vessels of the outer annual ring up and down from injection points in large, standing, leafy trees at relatively fast rates. These suspensoids usually ascended the first 5 feet in 8 to 15 seconds, reached a maximum distribution of 20 feet in less than 10 minutes, descended with like speed, and reached a maximum distribution of 36 feet in 6 minutes.

CONCLUSION

It may be concluded that the rapid invasion during the leafy season of the bole and crown of the American elm by the fungi herein discussed is due to the distribution of the spores of these fungi in the large vessels of the new annual ring by the sap stream.

LITERATURE CITED

(1) ARNDT, C. H.
1929. THE MOVEMENT OF SAP IN COFFEA ARABICA L. Amer. Jour. Bot. 16: 179–190, illus.

(2) BANFIELD, W. M.
1937. DISTRIBUTION OF SPORES OF WILT-INDUCING FUNGI THROUGHOUT THE VASCULAR SYSTEM OF THE ELM BY THE SAP STREAM. (Abstract) Phytopathology 27: 121–122.

(3) ———
1938. RELATION OF SAP FLOW, VESSEL LENGTH, SPORE DISTRIBUTION TO DEVELOPMENT OF DUTCH ELM DISEASE IN AMERICAN ELM. (Abstract) Phytopathology 28: 3.

(4) ——— and SMITH, A. L.
1936. GROWTH AND DISTRIBUTION OF CERATOSTOMELLA ULMI IN TISSUES OF ELM. (Abstract) Phytopathology 26: 86.

(5) BARY, A. DE.
1884. COMPARATIVE ANATOMY OF THE VEGETATIVE ORGANS OF THE PHANEROGAMS AND FERNS. 659 pp., illus. Oxford.

(6) BEDARD, W. D.
1938. CONTROL OF THE MOUNTAIN PINE BEETLE BY MEANS OF CHEMICALS. Jour. Forestry 36: 35–40, illus.

(7) BETREM, J. G.
1929. DE IEPENZIEKTE EN DE IEPENSPINTKEVERS. Tijdschr. over Plantenziekten 35: [273]–288, illus.

(8) BROEKHUIZEN, SIMON.
1929. WONDREACTIES VAN HOUT; HET ONTSTAAN VAN THYLLEN EN WONDGOM, IN HET BEZONDER IN VERBAND MET DE IEPENZIEKTE. 80 pp., illus. Leiden.

(9) BUISMAN, CHRISTINE.
1933. DIE IEPENZIEKTE. Nederland. Heidemaatsch. Tijdschr. 45: [219]–237, illus.

(10) ———
1936. VERSLAG VAN DE ONDERZOEKINGEN OVER DE IEPENZIEKTE, VERRICHT IN HET PHYTOPATHOLOGISCH LABORATORIUM WILLIE COMMELIN SCHOLTEN TE BAARN GEDURENDE 1935. Tijdschr. over Plantenziekten 42: [21]–44. [Abstract in Rev. Appl. Mycol. 15: 541–542.]

(11) CLINTON, G. P., and MCCORMICK, FLORENCE A.
1936. DUTCH ELM DISEASE GRAPHIUM ULMI. Conn. (State) Agr. Expt. Sta. Bul. 389, pp. 701–752, illus.

(12) COLLINS, C. W., BUCHANAN, W. D., WHITTEN, R. R., and HOFFMANN, C. H.
1936. BARK BEETLES AND OTHER POSSIBLE INSECT VECTORS OF THE DUTCH ELM DISEASE CERATOSTOMELLA ULMI (SCHWARZ) BUISMAN. Jour. Econ. Ent. 29: 169–176.

(13) CREAGER, DON BAKER.
1937. THE CEPHALOSPORIUM DISEASE OF ELMS. Arnold Arboretum Contrib. 10, 91 pp., illus.

(14) DIXON, HENRY H.
1924. THE TRANSPIRATION STREAM. 80 pp., illus. London.

(15) ——— and BALL, NIGEL G.
1922. TRANSPORT OF ORGANIC SUBSTANCES IN PLANTS. Nature [London] 109: 236–237.

(16) FRANSEN, J. J.
1931. ENKELE GEGEVENS OMTRENT DE VERSPREIDING VAN DE DOOR GRAPHIUM ULMI SCHWARZ VEROORSAAKTE IEPENZIEKTE DOOR DE IEPENSPINTKEVERS, ECCOPTOGASTER (SCOLYTUS) SCOLYTUS F. EN ECCOPTOGASTER (SCOLYTUS) MULTISTRIATUS MARSH. IN VERBAND MET DE BESTRIJDING DEZER ZIEKTE. Tijdschr. over Plantenziekten 37: [49]–61, illus.

(17) Fransen, J. J. and Buisman, Christine.
1935. infectieproeven op verschillende iepensoorten met behulp van iepenspintkevers. Tijdschr. over Plantenziekten 41: [221]–239, illus.
(18) Goss, R. W. and Frink, Paul Raymond.
1934. cephalosporium wilt and die-back of the white elm. Nebr. Agr. Expt. Sta. Res. Bul. 70, 24 pp., illus.
(19) Hales, Steph.
1727. vegetable staticks; or, an account of some statical experiments on the sap in vegetables . . . 376 pp., illus. London.
(20) Hopkins, A. D.
1894. black holes in wood. W. Va. Agr. Expt. Sta. Bul. 36, pp. [311]–336, illus.
(21) Huber, Bruno.
1935. die physiologische bedeutung der ring- und zerstreutporigkeit. Deut. Bot. Gesell. Ber. 53: 711–719.
(22) May, Curtis.
1934. outbreaks of the dutch elm disease in the united states. U. S. Dept. Agr. Cir. 322, 19 pp., illus.
(23) Middleton, William, Buchanan, W. D., May, Curtis, and Walter, J. M.
1935. ceratostomella (graphium) ulmi, the cause of the dutch elm disease, transmitted by scolytus multistriatus. Jour. Econ. Ent. 28: 138.
(24) Prell, H.
1930. ulmensterben und ulmenborkenkäfer ursachen und bekämpfung einer epidemischen baumkrankheit. Kranke Pflanze 7: 89–93, 103–105, 124–127.
(25) Preston, R. D.
1938. the contents of the vessels of fraxinus americana l., with respect to the ascent of sap. Ann. Bot. [London] (n. s.) 2: 1–21, illus.
(26) Priestley, J. H.
1935. radial growth and extension growth in the tree. Forestry 9: [84]–95.
(27) ——— Scott, Lorna I., and Malins, Marjorie E.
1933. a new method of studying cambial activity. Leeds Phil. and Lit. Soc. Proc. 2: 365–374, illus.
(28) ——— Scott, Lorna I., and Malins, Marjorie E.
1935. vessel development in the angiosperm. Leeds Phil. and Lit. Soc. Proc. 3: 42–54, illus.
(29) Radulescu, Theodor.
1937. beiträge zur kenntnis der baumkrankheiten. 2. versuche über das ulmensterben. Forstwiss. Centbl. 79: [629]–643, illus.
(30) Readio, P. A., Collins, D. L., Welch, D. S., Parker, K. G., and Tyler, L. J.
1937. the dutch elm disease. N. Y. (Cornell) Agr. Expt. Sta. Ann. Rpt. (1936) 49: 100–101.
(31) [Shevyrev, I.] Chewyreuv, Ivan.
1903. la nutrition extraracinaire des arbres malades. 49 pp., illus. Selsk. Khoz. Liesovodstvo 209: 58–103, 1903. St. Petersburg Imp. Soc. Nat. Conf. (1894), Bot. Div. Records. [Translation in U. S. Dept. Agr. Libr.]
(32) Shull, Charles A.
1924. imbibition in relation to absorption and transportation of water in plants. Ecology 5: 230–240.
(33) Smith, Fanny, Dustman, R. B., and Shull, Chas. A.
1931. ascent of sap in plants. Bot. Gaz. 91: 395–410, illus.
(34) Strasburger, Eduard.
1891. ueber den bau und die verrichtungen der leitungsbahnen in den pflanzen. Hist. Beitr. 3, 1000 pp., illus. Jena.
(35) True, R. P., and Slowata, Stanley S.
1939. scouting and sampling elms with symptoms commonly associated with the dutch elm disease as an aid in eradicating ceratostomella ulmi. Phytopathology 29: 529–537, illus.

(36) TUBEUF, [C. VON].
1935. WERDEGANG DER ERFORSCHUNG DER SOG. ULMENKRANKHEIT IN EUROPA VON 1921 BIS 1935. Ztschr. f. Pflanzenkrank. 45: [49]–78, [161]–189, illus.
(37) WENT, JOHANNA C.
1937. VERSLAG VAN DE ONDERZOEKINGEN BETREFFENDE DE IEPENZIEKTE, VERRICHT OP HET PHYTOPATHOLOGISCH LABORATORIUM "WILLIE COMMELIN SCHOLTEN" TE BAARN, GEDURENDE 1936. Tijdschr. over Plantenziekten 43: [75]–90.
(38) WOLLENWEBER, H. W.
1927. DAS ULMENSTERBEN UND SEIN ERREGER, GRAPHIUM ULMI SCHWARZ. Nachrichtenbl. f. den Deut. Pflanzenschutzdienst. 7: [97]–100, illus.
(39) ———
1929. DAS ULMENSTERBEN UND SEIN ERREGER GRAPHIUM ULMI SCHWARZ. Biol. Reichsanst. f. Land u. Forstw. Flugbl. 94, 4 pp., illus. [Also in Ztschr. f. Pilzk. 8: 162–166, illus.]
(40) ———
1929. DIE WIRTELPILZ-WELKEKRANKHEIT (VERTICILLIOSE) VON ULME, AHORN UND LINDE USW. Biol. Reichsanst. f. Land u. Forstw. Arb. 17: [273]–299, illus.
(41) ——— and STAPP, C.
1928. UNTERSUCHUNGEN ÜBER DIE ALS ULMENSTERBEN BEKANNTE BAUMKRANKHEIT. Biol. Reichsanst. f. Land u. Forstw. Arb. 16: 283–324, illus.
(42) YENDO, YASUTARO.
1917. INJECTION EXPERIMENTS ON PLANTS. Tokyo Imp. Univ., Col. Sci. Jour. 38, Art. 6, 46 pp., illus.

A HISTOLOGICAL STUDY OF SNAP BEAN TISSUES AFFECTED WITH BLACK ROOT[1]

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INTRODUCTION

In previous papers (*5*, *6*)[3] the writer described the symptoms and etiology of black root, a new disease of snap beans (*Phaseolus vulgaris* L.). Since vascular discoloration is such a prominent symptom of black root, it appeared that a further study of certain histological details of diseased plant parts would contribute to a better understanding of the effects of the disease on the physiology and vitality of the entire plant. The results of such a study form the basis of this paper.

Several investigators have reported the results of anatomical and histological studies on various plants affected with virus diseases (*1*, *2*, *4*, *9*). Others have reported disease symptoms on various plants, which, if studied histologically, would doubtless yield similar results (*7*, *10*, *11*). The remarkable degree of concurrence of opinion and observation on numerous critical phenomena found in virosed tissues supposedly caused by unrelated viruses, attest to the interest in work of this kind and suggest the ultimate fruitfulness of this approach to a better understanding of the interrelationship of plant and virus physiology; even, perhaps, to interrelationships of viruses themselves.

MATERIALS AND METHODS

Several varieties of snap beans, exhibiting various intensities of symptoms, in addition to several strains of beans originating from hybrid progenies, were used.

Both fresh and fixed materials were studied, the latter being killed in either Crafs fixative or formal-acetic alcohol, embedded in paraffin, sectioned and stained. Fresh material was used exclusively in free-hand sections, while the embedded material was cut 10μ to 15μ in thickness with a rotary microtome.

Various stains were used, but of these Pianese IIIb, alcoholic safranine counterstained with light green in clove oil, and Heidenhain's iron-alum haematoxylin proved best for the general purposes of this work. Certain details relating to suberin and cellulose deposition were confirmed microchemically by appropriate reagents. However, several points could best be determined from unstained sections which afforded an excellent check, particularly on the patterns of pathological cells.

With Pianese IIIb, healthy tissues stained various shades of magenta, except the middle lamellae of vessels, which stained green. The contents of diseased phloem and cambium tissues stained from light

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[3] Italic numbers in parentheses refer to Literature Cited, p. 690.

elm green to dull blackish green (*8*), depending on the severity of involvement and, apparently, on the length of time the elements had been undergoing degenerative changes. The contents of those elements that showed the earliest symptoms of disease usually stained the deepest green, while the walls always stained magenta. Likewise gum,[4] when present in vessels, stained green.

With safranine-light green, the cell walls and contents of healthy phloem and cambium tissues stained green, with the exception of the nuclei and slime bodies which stained red. Lignified walls of the xylem tissues likewise stained red. The cell walls and contents of diseased tissues, both phloem and cambium, stained from Pompeian red to dark hyssop violet. In fact, the degree and intensity of red stain in the walls of these tissues afforded a true index to pathological involvement, even before marked degenerative changes in the cell contents were evident.

With Heidenhain's haematoxylin, healthy cells stained lightly in various shades of blue to purple, while diseased tissues stained various shades of olive to almost black. Its use greatly facilitated the photographic work.

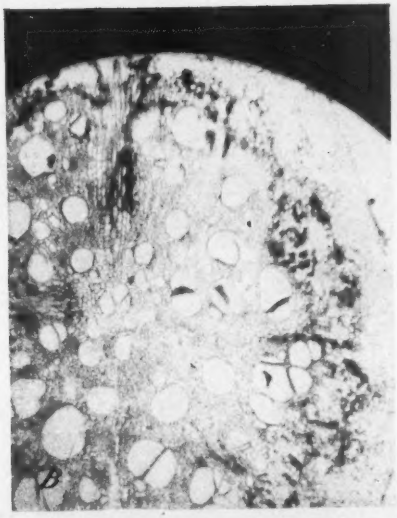
HISTOLOGY OF THE ROOT

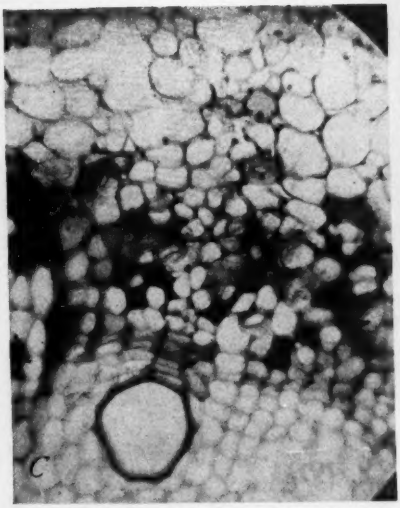
As stated in an earlier paper (*6*), the taproot of diseased plants never escapes infection. Exteriorly, infected roots exhibit a generalized dark to almost black color, depending on the severity of infection. It has been assumed that the discoloration is due to certain chemical changes attendant on the death of the protoplasm, perhaps oxidation, and no additional explanation has come to light from this investigation.

PHLOEM

In transverse and longitudinal sections of the diseased root (pl. 1), the visibly affected cells are seen to be confined almost exclusively to the phloem and cambium, although in certain cases discoloration is to be noted in the ray tissue and in the parenchyma surrounding the outermost layer of vessels. Under low magnifications all the tissues of a given region seem to be affected, but when higher magnifications are used (pl. 1, *B*, *C*, *D*), numerous areas showing no symptoms of disease are apparent. Conditions are readily demonstrable in given areas, particularly of the phloem in which the individual cells show symptoms ranging from isolated areas in portions of certain cell walls, that stain red, to those in which the cytoplasm and nucleus have undergone complete disorganization, now appearing as amorphous or gumlike inclusions that stain intensely red throughout (pl. 1, *B*, *C*). The young phloem fibers show symptoms of involvement, particularly during the early stages of differentiation. Judging from the reactions of the cell walls to the several stains employed and to Sudan III, IV, and Sudan black, it is fairly obvious that the cellulose walls of the phloem (including the fibers) have become either in part or completely covered by a deposit of suberin. Such a situation was demonstrated microchemically by Hill and Orton (*4*) in the course of histological studies on blue stem of potatoes. That the suberin is in some way correlated with protoplasmic disorganization is attested by the fact

[4] The term "gum" refers to the physical appearance of the substance, rather than to its chemical composition.





A-B, Transverse sections of roots showing distribution of black root symptoms; vascular discoloration is confined largely to the phloem and cambium, though in certain areas the rays and xylem parenchyma are discolored. *A* about ×6 and *B* about ×70. *C*, An area of *B* under higher magnification, showing details of cellular involvement. Note particularly certain initial stages of suberization in the walls of young sieve tubes and fibers directly above the complete xylem vessel. The walls of these elements are normally composed of cellulose. About ×750. *D*, Longitudinal section of a root showing symptoms of black root in the phloem and cambium. Slime bodies and the disorganized protoplasm show well, as do the suberized walls of diseased elements. Because they stain pale blue, the callose pads do not show well. About ×750.

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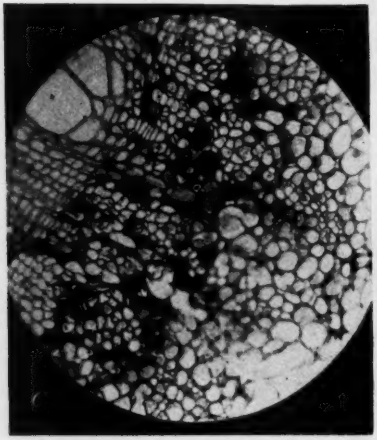
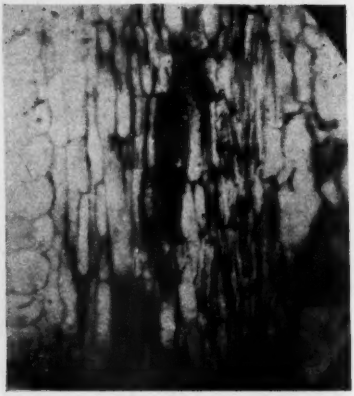
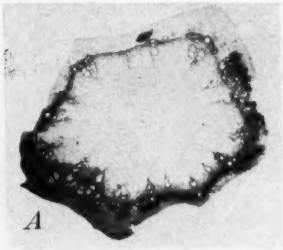
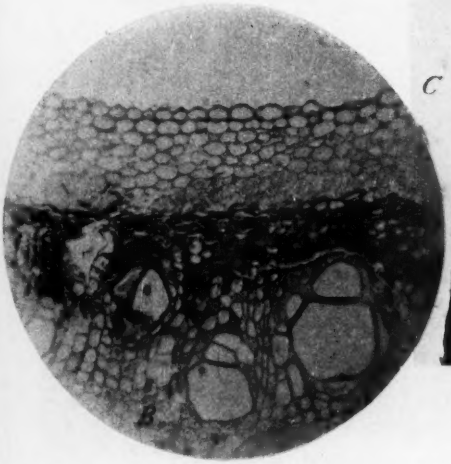


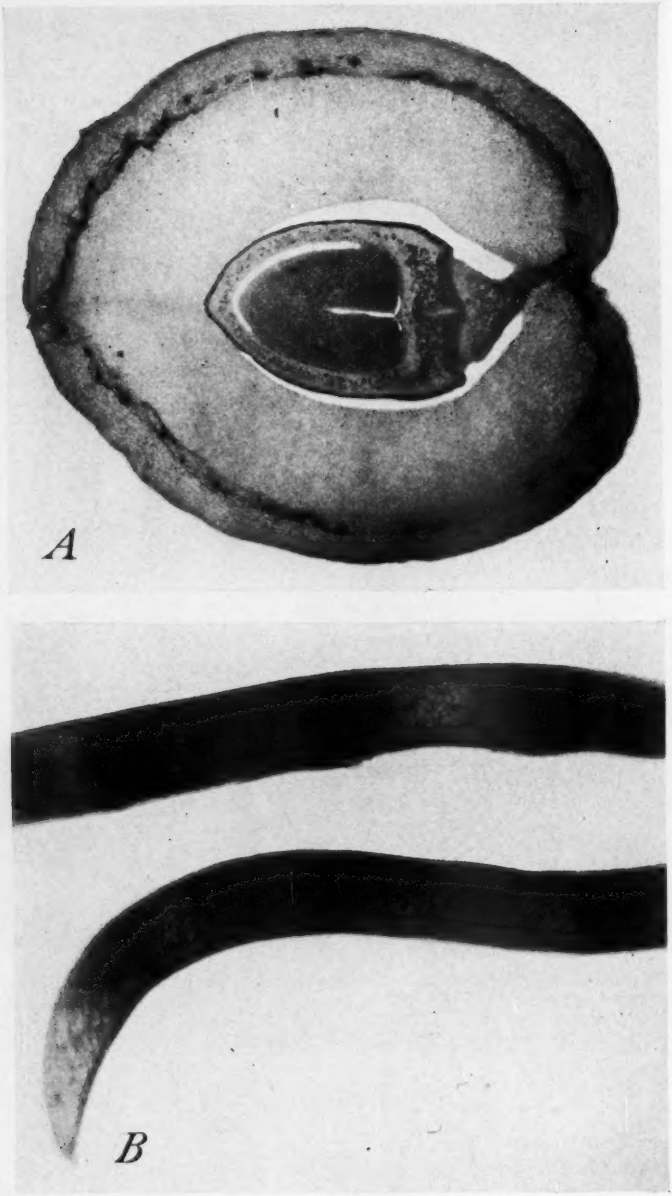


A-B, Transverse sections of the lower hypocotyl showing distribution of black root symptoms. *A* about ×6 and *B* about ×70. *C*, An area in *B*, under higher magnification showing details of cellular involvement. Note particularly the collapse of cells near the cambium, the walls of which are not heavily suberized. In other areas the intensity of discoloration makes it appear that cellular structure has been obliterated. About ×400. *D*, Longitudinal section of an area of the lower hypocotyl showing details of cellular involvement in the cambium and phloem. Here again callose pads are indistinct due to their staining reactions. About ×400.





A-B, Transverse sections of the above-ground stem showing the distribution of black root symptoms. The phloem, except for mature fibers, and the cambium are completely necrosed. *A* about ×25 and *B* about ×70. *C*, Tangential section through the stem showing early symptoms of black root. About ×750.



A, Transverse section of a pod in the snap bean stage showing distribution of black root symptoms. About ×16 (or ×25). *B*, Unstained, diseased bean pods, cleared to show the extent of vascular discoloration. About ×5.

that the deposits grow in thickness as the protoplasm degenerates until finally, in later stages, the walls are quite thick. Certain cells with less heavily suberized walls near the cambium are completely collapsed due perhaps to growth stresses of the surrounding healthy tissues, but no evidence of dissolution of cell walls has been found. The necrosed areas are notably lacking in starches, proteins, and fats, as compared with the surrounding, apparently unaffected areas.

In longitudinal sections, particularly of the phloem, several additional features can be seen to advantage. Normal cell constituents such as nuclei and slime bodies (Schleimklumpen of Strasburger) are particularly evident in the normal-appearing cells. In the diseased cells various degrees of involvement, as exemplified by protoplasmic disorganization, are evident (pl. 1, *D*). In certain sieve tubes, some of which show no evidence as yet of protoplasmic disorganization, callose pads are frequently found blocking the pores of the sieve plates. Sieve tubes thus affected recall a similar situation in perennial plants in which callose pads normally are present in the sieve tubes during the dormant period (*3*). Since the bean plant is an annual and does not, therefore, normally undergo a dormant phase, the writer is inclined to attribute the presence of callose pads in its sieve tubes to the activity of the virus. That no dormant phase normally exists in beans during the period in which the material was collected was obvious from examination of normal tissue. Physiologically, the presence of callose pads must greatly influence the transport of vital substances through the phloem. It could not be determined whether their formation precipitates the later-appearing degenerative phenomena or whether they are but coincidental with the beginning of a series of irreversible reactions that ultimately result in complete protoplasmic disorganization in affected cells. Chain reactions would seem to play a part in the sequence of events.

CAMBIUM

In all instances in which the phloem shows disease symptoms, the contiguous cambium likewise is affected. This generalization holds true not only for the root but for all portions of the plant in which a functional cambium has been found. Staining reactions identical with those described for phloem are evident in diseased portions of cambium. This is strictly true, however, only insofar as the meristematic zone immediately adjacent to the phloem can be regarded as cambium. The writer fully realizes the difficulty of defining a single row of meristematic cells as cambium to the exclusion of meristematic gradients contiguous on one side with phloem and on the other with xylem. However, those cells, not yet differentiated, lying closest to the diseased phloem show all gradations of suberization of cell walls and protoplasmic degeneration. Those nearest the phloem show symptoms identical with those of phloem, while those nearest the xylem exhibit symptoms typical of diseased xylem elements. In most instances, the cambial derivatives appear to undergo fewer divisions and to begin to differentiate much earlier in diseased areas than in areas relatively or entirely free from symptoms. Many cells with heavily suberized walls remain distinct, while others, usually showing deposits of less thickness, undergo almost complete collapse.

XYLEM

Transverse and longitudinal sections of xylem tissue show very few symptoms of disease except, as stated above, in the ray parenchyma and in the xylem parenchyma immediately adjacent to certain vessels nearest the cambium. In a few instances, gum has been demonstrated in the lumen of certain young vessels, but this is exceptional. Likewise, very few tyloses were seen. Suberin deposits are present on the walls of badly diseased xylem parenchyma. The contents of diseased xylem and ray parenchyma show all gradations of disorganization and gumlike inclusions, just as in the phloem (pl. 1, *B*, *C*). The vessels are scalariform (as can be seen in longitudinal section) (*3*). In many instances the pores in a given view were definitely plugged by small deposits of gum, but no evidence is available to indicate how extensively the pores in a given vessel, or in a series are plugged. However, as will be brought out later, it is hardly necessary to find or postulate complete vessel plugging to account for the failure of the xylem as a conductive system.

HISTOLOGY OF THE HYPOCOTYL

For the purposes of this study the hypocotyl is regarded critically, for it is in this area that practically all the transition of the vascular arrangement from root type to stem type takes place. Since there is, relatively, a greater amount of differentiated vascular elements in the hypocotyl than in either root or stem, it is not surprising that one finds the diseased areas more conspicuous here and more uniformly distributed than in either the root or stem.

Here, however, the necrotic areas are confined for the most part to the phloem and cambium, as was true in the root. Instances of ray penetration and involvement of xylem parenchyma are evident, but proportionately about as in the root (pl. 2). In several instances it appears that the cambium may be killed more quickly than the root.

Individual diseased cells, whether in phloem, cambium, or xylem, give identical staining reactions and distribution as do like cells from the root.

HISTOLOGY OF THE STEM

As noted in a former publication (*6*), black root symptoms on the stem are not uniform, but reveal their presence by a dark streak or streaks running lengthwise of the stem. Other symptoms such as wilting, chlorosis, and mosaic patterns, in the leaves are also present, but since these are common to several virus diseases of beans, stem streaking is regarded as a better diagnostic symptom.

Transverse and longitudinal sections show relatively smaller numbers of necrotic areas in the stem than are present in either root or hypocotyl; but closer inspection indicates that relatively more necrotic cells occur in given areas of the stem than in comparable areas elsewhere (pl. 3). It also appears that protoplasmic disorganization is more sudden and complete in the stem than elsewhere, as fewer transitional stages are to be seen in tissue of comparable age, and, supposedly, comparable as to the time of initial infection.

The distribution of necrotic areas and the completeness of protoplasmic disorganization in areas of the stem are definitely correlated with the percentage of leaves and fruiting pedicels that individually show no symptoms of disease for various periods of time after other

parts are wilted and dead. Whether initial infection of a given plant occurred through the above-ground parts or through the roots could not be ascertained from the present study. The histological evidence indicates, however, that the transitional area of the hypocotyl has an important relationship to the amount and distribution of necrosis in both the stem and root.

However, the individual necrotic areas of the stem, like those of the root and hypocotyl, are confined almost exclusively to the phloem and cambium. Nevertheless, as in other organs of the plant, protoplasmic disorganization often extends for short distances down the rays and into the xylem parenchyma surrounding the vessels contiguous to the cambium. Here, as elsewhere, certain vessels contain various amounts of gum, and the question arises again as to how completely the scalariform perforations are plugged.

HISTOLOGY OF THE POD

Although the only exterior symptoms of black root on the pods consist of a slight darkening of one or both sutures, accompanied by a slight pitting of the epidermis in older pods (*6*), transverse sections show that necrosis of the vascular system is quite extensive (pl. 4, *A*). The largest vascular bundles of the pod lie below the sutures. No doubt the size of these suture bundles, with their greater number of phloem elements, rather than the distribution of necrotic areas, is responsible for the expression of symptoms exteriorly only along the sutures. It is evident that essentially all the vascular tissue shows necrosis, but since the smaller bundles contain so little phloem, the intensity of discoloration is not sufficient to show through the chlorenchyma of fresh pod walls. It is thought, however, that the involvement of the smaller bundles is responsible for the pitting of the pods, as the pits appear to be the result of collapse of desiccated tissues, particularly in the vicinity of stomata. When the chlorophyll is extracted and the pods appropriately cleared, the extent of vascular necrosis is evident even in unstained preparations (pl. 4, *B*).

Phloem necrosis also extends from the ventral vascular (suture) bundles down through the funiculi into the seed. Evidence of suberization of cell walls and protoplasmic disorganization is evident to some extent in certain parenchyma (transfusion) areas along the smaller veins. In no instance was there evidence of vascular necrosis in the embryo, although considerable areas were found in the seed coats at the chalaza. This observation raises an interesting point in regard to the mechanics of seed transmission of black root. It is, of course, conceivable that the virus might pass through the transfusion areas of the seed coat at the chalaza and thereby reach the vascular system of the embryo. It is also conceivable that embryo infection takes place during germinative stages of the seed, since the enlarging embryo in rupturing the seed coat might release sufficient inoculum from the phloem of the seed coat to bring about infection. This viewpoint presupposes that the virus remains active in the seed coat, a point that has not as yet been investigated. However, we do know that under certain conditions a small percentage of the disease is seed borne (*6*). Both these hypotheses are contrary to findings of other investigators (*2*), but they were working with a disease which is not seed transmitted.

DISCUSSION

Plants affected with various virus diseases have been studied anatomically but of those characterized by necrosis, the reports on location, pattern, and appearance of the areas are rather uniformly in agreement as to certain critical details (*1*, *2*, and perhaps *4*).

Results of the present study have afforded an histological explanation for the odd distribution of symptoms produced by black root on snap beans. Apparently being confined largely to the phloem and cambium of the root, hypocotyl, stem, and pods of a given plant, the number of necrotic areas present seems definitely correlated with the amount and extent of symptoms that the plant exhibits. This observation seems particularly well borne out in the hypocotyl, where invariably the amount of necrotic tissue is relatively greater than in either root or stem of the same plant.

The limited extent to which the xylem tissue is involved and the fact that extensive gum or tylosis formation does not occur in the vessels indicates that factors other than necrosis are responsible for the rapid wilting of diseased plants in the presence of abundant moisture. Aside from the plugging of the sieve tubes by callose pads, which doubtless influences the nutrition of the plant, the writer feels that sudden wilting can best be explained histologically through the reaction of the virus on the xylem parenchyma. Even though only the parenchyma surrounding the outermost vessels, i. e., those contiguous to the cambium, is apparently affected, the result is the effectual and sudden wilting of the plant. This explanation can best be accepted by postulating the existence of a very rapid functional gradient between the old vessels and those that have recently differentiated (*3*), as well as assigning to the living xylem parenchyma an important influence on water conduction. Such an interpretation apparently was considered by Weimer (*9*) in his work on alfalfa dwarf. He found that when relatively inconsiderable numbers of xylem vessels contiguous to the cambium were plugged in the alfalfa roots the plants wilted. By pumping water through diseased and healthy roots he demonstrated that very little water passed through those roots in which the xylem vessels contiguous to the cambium were plugged. It is also conceivable that injury to the xylem parenchyma was a considerable factor in the wilting of the living alfalfa plants.

The writer is also not unmindful of the possibility that the vessels might be plugged by small deposits of gum over the scalariform perforations between vessels, as mentioned earlier in this paper. This could be a particularly significant factor when one considers the great amount of anastomosis of the ducts, particularly in the transition areas of the hypocotyl. It is very difficult histologically to demonstrate with certainty the proportion of the scalariform perforations that are plugged by gum.

Finally, the physiological effects of the amount and severity of phloem and cambium necrosis alone, without regard to the xylem, could well upset the nutritional balance of the plant to the extent that sudden wilting would result. Elaborated foods are essential to a wide variety of energy relations concerned in the necessarily continuous growth and extension of the root system into its "absorption environment," as well as to the actual process of material absorption from the soil. Theoretically, the root system could operate fairly efficiently

under the stress of diminishing food supplies for various periods of time; but when it is no longer possible for its compensation mechanism to maintain functional equilibrium, the root system fails and the plant suddenly wilts. Histological evidence for this explanation is necessarily fragmentary, consisting chiefly of tests for proteins, fats, starch, etc., all of which were much depleted or absent in necrosed tissues. This, too, is in accord with the findings of Hill and Orton (*4*).

In regard to the histology of seed transmission of black root, it can be said only that symptoms of infection (necrosis of phloem elements) have been traced by the writer into the seed coat near the chalaza. Other workers (*2*) found the same situation in their work with curly top of beets. It so happens that curly top is not transmitted through the beet seed, while black root is seed-transmitted, at least to some extent. Others have shown that relatively few virus diseases are seed-transmitted, the outstanding example of positive transmission being common bean mosaic. The histological evidence being the same for a disease that is sometimes transmitted through the seed (black root) as for one which is apparently never transmitted through the seed (curly top), it can be concluded only that some factor unknown to the writer determines the mode and frequency of embryonic inoculation. Hypothetically, it is reasonable to assume that inoculation might occur when the vascular system of the seed coat is ruptured during germinative stages of the seed.

SUMMARY

Snap beans affected with black root have been examined histologically. This report is based on studies of both fresh and fixed material, appropriately stained, of several varieties and progenies from segregating hybrids.

The peculiar external manifestation of such symptoms as differential streaking and wilting of certain plant parts has been shown to be correlated histologically with differential necrosis of the phloem, cambium, and outermost layer of xylem in the root, hypocotyl, stem, and pod.

Necrotic symptoms in the phloem varied from slight cytoplasmic disorganization in those cells recently infected through stages of more or less complete destruction of the cytoplasm and nucleus. In the end phases the cell contents resembled gum, and the cellulose cell walls became covered by deposits of suberin. Callose pads were also noted in sieve tubes showing early symptoms of disease. Whether the effect of these initiate degenerative changes or are but one of a series of chain reactions, is not known. In that area of the cambial zone contiguous to the phloem conditions similar to those in phloem prevailed; while in the areas nearest differentiating xylem, the symptoms most nearly resembled those characteristic of xylem. In the vessels very little evidence of plugging was observed except in the scalariform perforations, and very few tyloses were seen. The parenchyma about the youngest vessels contiguous to the cambium was severely necrosed, and necrosis was occasionally found in the ray parenchyma.

It is tentatively suggested that the sudden wilting characteristic of black root may be due in great part to cessation of activity of the young vessels through destruction of the vital xylem parenchyma. The possibility of mechanical blocking of the scalariform perforations

by gum, particularly in the hypocotyl, as well as the probable effect of phloem and cambium necrosis on the energy relations of the root system, are also considered.

Necrosis of the phloem was observed to extend throughout the vascular system of the pod and through the bundles of the funiculi into the seed coat at the chalazal end of the seed. No evidence of infection was observed in the embryo, but only very young embryos were examined. It is suggested that seed transmission may be accomplished by transfusion of the virus from the seed coat to the embryo, or that inoculation may occur as the embryo ruptures the vascular system of the seed coat during germination.

LITERATURE CITED

(1) ARTSCHWAGER, ERNEST, and STARRETT, RUTH C.
1936. HISTOLOGICAL AND CYTOLOGICAL CHANGES IN SUGAR-BEET SEEDLINGS AFFECTED WITH CURLY TOP. Jour. Agr. Res. 53: 637–657, illus.

(2) BENNETT, C. W., and ESAU, KATHERINE.
1936. FURTHER STUDIES ON THE RELATION OF THE CURLY TOP VIRUS TO PLANT TISSUES. Jour. Agr. Res. 53: 595–620, illus.

(3) EAMES, ARTHUR J., and MACDANIELS, LAURENCE H.
1925. AN INTRODUCTION TO PLANT ANATOMY. 364 pp., illus. New York.

(4) HILL, L. M., and ORTON, C. R.
1938. MICROCHEMICAL STUDIES OF POTATO TUBERS AFFECTED WITH BLUE STEM DISEASE. Jour. Agr. Res. 57: 387–392, illus.

(5) JENKINS, WILBERT A.
1939. A NEW DISEASE OF SNAP BEANS. Science (n. s.) 90: 63.

(6) ———
1940. A NEW VIRUS DISEASE OF SNAP BEANS. Jour. Agr. Res. 60: 279–288, illus.

(7) NEILL, J. C., BRIEN, R. M., and CHAMBERLAIN, E. E.
1934. "SORE SHIN": A VIRUS DISEASE OF BLUE LUPINS. New Zeal. Jour. Agr. 49: 139–146, illus.

(8) RIDGWAY, ROBERT.
1912. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C.

(9) WEIMER, J. L.
1937. EFFECT OF THE DWARF DISEASE ON THE ALFALFA PLANT. Jour. Agr. Res. 55: 87–104, illus.

(10) WHIPPLE, O. C., and WALKER, J. C.
1938. TWO STRAINS OF CUCUMBER VIRUS ON PEA AND BEAN. (Abstract) Phytopathology 28: 22.

(11) ZAUMEYER, W. J.
1938. A STREAK DISEASE OF PEAS AND ITS RELATION TO SEVERAL STRAINS OF ALFALFA MOSAIC VIRUS. Jour. Agr. Res. 56: 747–772, illus.

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